13th Annual NIH SBIR/STTR Conference
Poster Abstracts

1

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Safety Aspects and Drug Delivery Performance of Visulex® Passive Delivery System

Objective: 1) To establish ocular safety of weekly Visulex® applications of four different dexamethasone sodium phosphate (DSP) formulations for 12 weeks in rabbit; 2) To study DSP distribution in the ocular tissues after a single dose of Visulex® application.

Methods: The Visulex® passive drug delivery system for ophthalmic usage features a fast swelling matrix with quick absorption and release that is suitable for both hydrophilic and hydrophobic drugs. It is easy to apply and remove, self-adheres to the eye during the application, and provides corneal protection. For these studies, the dimensions of the Visulex® applicator were altered to fit a mature New Zealand white rabbit (NZW), but all other features were kept the same as the Visulex® human applicator. All experiments were performed with NZW rabbits. Four formulations of DSP were tested. A Visulex® applicator loaded with each formulation was applied to the eye with the drug matrix in immediate contact with the conjunctiva/sclera for 5 to 20 min, and then the applicator was removed. For the drug distribution study, the rabbits were sacrificed immediately after device removal. The eye was dissected into seven tissue sections (i.e. anterior chamber, lens, retina-choroid, cornea, vitreous, conjunctiva, and sclera). The total amount of DSP in the tissues was determined by HPLC. For the safety study, the rabbits received weekly dosing for 12 applications. The eyes were examined daily with an indirect ophthalmoscope and graded based on a modified McDonald-Shadduck grading scale for discharge, conjunctival irritation, corneal defect, synechia, hypopion, anterior chamber flare, and vitreal opacity. At the end of the 12 weeks, the animals were sacrificed, the eyes collected, and histopathological examination performed.

Results: In the drug distribution study, significant amounts of DSP were found in anterior chamber (6-48 µg), retina/choroid (6-52 µg), cornea (14-174 µg), vitreous (4-15 µg), conjunctiva (35-241 µg), and sclera (52-265 µg). The total amounts of DSP in the eye were 121-764 µg and are directly proportional to the concentration of DSP in the formulation and upon the duration of the Visulex® applicator's contact with the eye. For the ocular safety study, the weekly Visulex® treatments were well tolerated over a 3 month period. Minimal conjunctival injection and swelling were observed immediately after dosing in all groups, which resolved in 1 to 7 days. It appears that signs of irritation correlate to both DSP concentration and application duration. There were no corneal or lens opacities observed. There was no irreversible ocular tissue damage observed by either macroscopic observation or histopathological examination.

Conclusions: 1) Visulex® applicator for human use has been developed and tested for safety and drug delivery performance in rabbit. 2) 3-month safety study suggests Visulex® containing DSP formulation is well tolerated in rabbit with no sign of irreversible ocular tissue damage. 3) Visulex® can deliver significant amounts of DSP into the eye tissues, including the sclera and retina/choroid, and shows potential for use in transscleral drug delivery.

2

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Demand Driven Healthcare Staff Scheduling using Flexible Shift and Monte-Carlo Simulation

The shortage of nurses and medical technologists is accelerating. Shortages can be reduced by scheduling staff to precisely meet the *hour-by-hour demand for medical service*. Think of bank teller scheduling, where more staff are scheduled at peak demand.

Current attempts to schedule to demand are relatively primitive: a small number of quantized fixed shifts, for example: 7am-3pm, 10am-6pm, 3pm-11pm, 7am-7pm, 7pm-7am, etc. is allowed. The pre-determined fixed shifts are rigid *input* parameters for the scheduling process and workers are assigned to the shifts.

In this innovation, fixed shifts are replaced with *flexible shift parameters*; specifically, a range of start times and shift durations that are harmonious with worker lifestyles. These parameters become elastic *inputs* for the scheduling algorithm. The actual shift assigned to a worker on any particular day is computed with the objective to have just enough workers to meet the hour-by-hour demand.

Small Business Innovation Research (SBIR) Phase I research successfully determined the efficacy of worker-friendly, flexible shift scheduling and found savings of 4% are possible. Four percent can cut the current worker shortfall significantly and corresponds to annual savings of $3.5 billion in healthcare costs. Despite many scientific studies of flexible shift scheduling, there is a dearth of practical commercial applications primarily due to the complexity of technologies employed in the research. In SBIR Phase II, a simple but powerful technology, Monte-Carlo simulation, is being employed.

The hypothesis is that a Monte-Carlo simulation can be developed that uses worker-friendly, flexible shift parameters to precisely meet the hour-by-hour demand for medical service. The specific aim is to develop a Monte-Carlo scheduling algorithm in which flexible shift parameters are *inputs* and the actual shift assigned to a worker is *computed* during the scheduling process.

Results through the first half of SBIR Phase II research will be presented.

The Demand Driven software being developed is being integrated into, and will become a new module for, DOCS Scheduler software, an established healthcare staff scheduling software package being used by scores of healthcare providers. The Demand Driven module will add a distinguishing competitive advantage to DOCS Scheduler. Business model simulation predicts a Phase III break even at month 24. DOCS Scheduler, with the new Demand Driven module, received a Merwyn Business Simulation concept score of 42, a strong indication of commercial success.

3

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Novel Bioprocess Platform for Rapid, Economical Production of Difficult Peptides and Proteins

Objective: To develop and optimize methods for synthesizing large peptides and small proteins that are difficult, excessively costly, or environmentally damaging to synthesize by existing methods.

Methods: A novel bioprocess platform that synthesizes recombinant peptides or proteins as a C-terminal extension of an optimized UBL (ubiquitin-like protein) and then employs a UBL-specific protease to release the peptide. The system was optimized for maximum expression level in bacteria (E. coli), purification, stability and *in vitro* cleavage kinetics for over a dozen product candidates. Histidine tags facilitate initial purification of the UBL fusion protein using immobilized metal chelation chromatography and subsequent removal of the UBL and protease from the reaction mixture. The specificity of the protease is exquisite. No exopeptidase activity has ever been observed and the peptide products are recovered intact. Our recombinant UBL protease is also extremely robust. Cleavage reactions typically approach 100% completion in a few hours at 37°C. The UBL itself is resistant to degradation by bacterial proteases and can accumulate to up to 70% of the total intracellular protein.

Results: Preliminary studies and cost projections suggest that peptides ranging from about 25 to 70 amino acids can be produced for dollars per gram, in contrast to chemical synthetic methods for which costs and lead times dramatically increase with the length of the peptide product. The ability to produce the final peptide without many closely related impurities such as those generated in chemical synthesis reduces the purification task which is a major component of the high cost of chemical synthesis. Difficulties with host toxicity and genetic instability of the target protein, as well as purification issues, have been overcome for specific product candidates, using the platform.

Conclusion: The platform was developed and proof of concept that the platform could be a greener, more cost effective, and more rapid alternative for synthesis of large peptides and small proteins or protein domains was obtained. APC Biotech has performed several feasiblity studies with the platform for clients with difficult peptides, proteins, and protein domains and is in the process of licensing the platform to one of its customers. APC is also using the platform to manufacture its own products, including veterinary vaccines and biopharmaceuticals. Licensees are sought.

4

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Cost Effective Production of Therapeutic Recombinant Antibodies in Saccharomyces Cerevisiae

ApoLife, Inc. is a privately held biotechnology company with a proprietary yeast based protein production platform for accelerated development of monoclonal antibodies and protein therapeutics. ApoLife is using its platform for co-development of novel proteins for cancer therapy with NCI and seeking additional research partnerships.

ApoLife's proprietary system includes "Twin Cassette" vectors which enables cloning of two chains of antibody into a single vector, which facilitates co-expression of both chains. It also includes genetically engineered yeast strains for high level of secretion of complex proteins. The preliminary data shows no hypermannosylation of antibody produced form our yeast. ApoLife has licensed yeast strains bioengineered to produce human like glycosylation of antibodies. The platform has been validated to produce antibodies, antibody fragments, fusion proteins and proteins that are difficult to produce or cannot be produced in any other expression system. The advantages of yeast over current mammalian cell culture include rapid growth to high density in inexpensive media, shorter fermentation time of 7days versus 21 days in mammalian cells and products are free of animal viruses and endotoxins.

ApoLife has constructed Twin cassette vector for Campath IgG genes which are under the control of alcohol dehydrogenase promoter, and α-mating factor secretion signal sequence. The proprietary *S. cerevisiae* strains transformed with Campath IgG genes secrete intact antibody into the culture medium. The codon optimization and selected promoter sequences helped to improve the yield of Campath

antibody into media and this data will be presented. (NCI SBIR Contract HHSN261201000096C, 09/30/2010).

ApoLife is also developing novel strategy for production of secretory sIgA for mucosal therapy of *C.difficile* infections. The complex structure of sIgA requires expression of 4 genes in a single cell. ApoLife's innovative approach is to employ, the "Twin Cassette®" plasmid, combined with the integration of 2 genes in *S.cerevisiae* yeast genome. This will facilitate simultaneous expression of four genes for sIgA in a single yeast cell making the system efficient for production of tetravalent sIgA for mucosal immunotherapy. This strategy can be applied to develop sIgAs for treating other infectious diseases like colitis.

ApoLife's business strategy is to develop biogeneric and proprietary antibody and protein products for cancer and infectious disease therapeutics and diagnostics through corporate partnerships and licensing. ApoLife's platform is expected to shorten the discovery phase for antibodies by 30%, reduce the manufacturing capital cost by 50%, and most importantly produce antibodies with better therapeutic effect which will lower the healthcare costs. ApoLife is actively seeking strategic partnerships by offering research licenses to companies or scientists from research institutes.

5

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Capitalization of Investing Into Medical Device Development on the Health Care Market

Medical Device is a tool (product and application) intended to fill unmet needs of health care providers (customers) and their patients (consumers) on the health care markets. It means that the final capitalization of the medical device investment takes place during the trade between the producer/seller and the customer/consumer at the market.

Consequently, the investment into medical device development must be planned as a function of the number of units sold and the individual unit's price on the market plus the expected profit per unit. The other part of the equation is the pre-sales cost. This cost includes, but is not limited to: (1) Manufacturing cost (idea, prototype, testing, product manufacturing), (2) Regulatory cost (approval for sales in individual countries), (3) Wholesale cost (warehouse, gross sales, brokering sales); (4) Export/import licenses, (5) Shipping and Handling, (6) Distributor's cost, (7) Retailer's cost, (8) Customer's cost, (9) Taxes on production, marketing and sales, and (10) Market research and product marketing cost.

This is the Capital Cycle, a model for product capitalization which is the most profitable; but requires large investment into product development (R&D + Regulatory) and business infrastructure to carryon marketing and sales. Only large companies may opt for this model.

Small businesses have two options: (1) Licensing Intellectual Property and (2) Selling (acquisition/merger) of the company. Both are the Capital Exit models, which can be applied at any time during the pre-sale product development process and both carry the same danger of negative consequences to the product and the intended goal to meet public needs. The benefits of this product could be lost or postponed. Fast money and early exits promoted by investors and/or shareholders could be the main reason why many potentially important medical devices never reached the market.

Usually the small business founders prefer licensing (Angel investors or corporate), and the small business companies regard the Early Exit Strategy as preferred solution for their investment (venture

capital and corporate acquisition/merger). As the device development progresses, the small business founders find themselves in a chronic lack of cash and under pressure to make many compromises—some of which are causing delays of the development or change in the device design.

This poster will be passed upon a real success story of one diagnostically potent biomarker and the companies built around it to fund its pathway to the health care markets.

6

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Novel Neuroreparative Therapy for MS That Promotes Remyelination

Cognosci has been dedicated to develop novel anti-inflammatory and neuroreparative therapies for major neurological disorders including multiple sclerosis. Multiple sclerosis is one of the most common neurological diseases in young adults that is pathologically characterized by inflammation and demyelination in the brain and the spinal cord accompanied by axonal damage. The current FDA-approved medicines for MS specifically target the inflammatory phase of the disease, but they have no clear activity in myelin or axon repair. Cognosci has developed the “COG” series of compounds with therapeutic potential for MS that display a useful combination of anti-inflammatory, neuroprotective and neurorestorative activities.

Thanks to NINDS’s funding for both Phase I and II SBIR grants, we have successfully identified a lead compound COG112 for treatment of multiple sclerosis through an in vitro screening platform and several in vivo animal models of human multiple sclerosis. Specifically, when administrated after disease onset, COG112 demonstrated significant therapeutic efficacy by reducing overall disease severity and promoting recovery from clinical symptom in both myelin oligodendrocyte glycoprotein- and proteolipid protein-induced experimental autoimmune encephalomyelitis models in mice. The improved function following COG112 treatment is also accompanied with reduced demyelination in spinal cord and reduced peripheral T cell infiltration and macrophage activation. These data suggestion that COG112 can efficiently suppress inflammatory response and prevent the brain and spinal cord from inflammatory damage in animal models of multiple sclerosis.

In addition to its anti-inflammatory activity, we also obtained proof-of-concept that COG112 can beneficially promote myelin reconstruction and axonal regeneration in multiple in vivo and in vitro models, and can help to preserve myelin-forming cells, i.e., oligodendrocytes and their precursor cells. In an in vitro model of demyelination induced by lysolecithin on postnatal rat cerebellar slice cultures, COG112 significantly increased the synthesis of myelin basic protein (MBP), indicating remyelination was promoted by COG112. Similarly, COG112 also promoted remyelination in vivo in a cuprizone-induced demyelination model in mice. Using primary neuronal cultures or PC12 cell line, we found that COG112 can promote axon extension and overcome inhibition of axon growth by myelin or Nogo-A, which are the major inhibitors of axon regeneration following demyelination in multiple sclerosis. Using a sciatic nerve crush model in mice, we also obtained data supporting that COG112 facilitates regeneration and remyelination of peripheral nerves.

Taken together, all the data support that COG112 represents a potential therapy for multiple sclerosis which possesses triple activities, i.e., inhibiting inflammation, promoting remyelination and promoting neuroregeneration. These are all most desirable traits for multiple sclerosis therapy. Based on the success of SBIR-funded early stage of development, we are going to conduct preclinical safety/toxicity study required for an Investigational New Drug application to the Food & Drug Administration (FDA). Once approved by the FDA, Cognosci will proceed to initiate human Phase I, Phase II and Phase III clinical trials and eventually move COG112 into the market to benefit multiple sclerosis patients.

7

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Novel Pharmaceutical Solvent Water Separation System

Specific Aim: There are numerous needs in the pharmaceutical industry to produce or recover high purity solvents. Key uses include: high purity alcohol for various applications, the ability to develop low-cost dewatering of solvents and a relative gentle and simple process for dewatering solvent and concentrating biologicals under mild conditions. Compact Membrane Systems (CMS) developed a novel membrane process that has lead to extremely high dewatering rates with high separation capabilities based on a family of chemically inert membranes operating under a wide range of operating conditions. CMS's dewatering process is compatible with existing pharmaceutical solvent (PS) processing. While traditionally more chemically active membranes have been used in liquid pervaporation (PV) mode, CMS has identified and provides data that suggests the unique separation capability by operating the system in either liquid/vapor PV mode or vapor/vapor mode. CMS dewatering system is based on inert membranes and has relatively universal applicability. Therefore, from a chemical stability standpoint, it can be operated with alcohols, organic acids, ketones, amines and aprotic solvents, to name a few. Since CMS membrane's high flux is based on its high free volume and inert nature, there is little need for concern about chemical interaction with the species present, and the actual permeability does not change significantly with water activity. Therefore, we have a universal simple system that can work on a wide range of PS for a wide range of applications under varying water activity.

Product Concept: CMS in combination with industrial partners have fabricated a composite membrane for drying pharmaceutical solvents (PS). The top thin dense selective layer will be made from an inert polymer which has very high (and stable) water transport even at low water activity. The support is preferably a hollow fiber (HF) with excellent chemical resistance, small pores on the membrane side for good support and an open structure behind the pores for minimal resistance.

Innovation: Membrane inroads into PS dehydration of solvents in the pharmaceutical and related industries has been extremely modest. Polyvinyl alcohol (PVA) membranes are a product of the market today, but it has significant temperature and chemical limitations. Silica membrane also has significant chemical limitations. An inert membrane with exceptional water-organic separation capabilities is a truly unique, innovative and valuable product. A high water vapor / organic separation provides for a high recovery and efficient operation with the inert nature, it will have universal applicability for almost any water organic environment.

Product Enhancements: Often pharmaceutical catalytic processes can be poisoned by water, oxygen and other contaminants. CMS has demonstrated that by removing these contaminants through the CMS membrane that catalyst poisoning can be kept to a minimum.

Poster: Will show actual pilot and laboratory results done using commercial modules available for customer use.

8

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Development of Oncomine for Biopharmaceutical Research

In 2007 Oncomine was a novel cancer genomics database that demonstrated the utility of aggregating published cancer genomic data and making it widely available to academic users via a web-based interface. However, the underlying software architecture was not structured to meet the demands of the commercial market in terms of stability, scalability, and clinical utility. **Thus the overall objective of this study was to develop Oncomine for commercial use by making structural changes to the data pipeline, database architecture, and user interface.** The Oncomine data pipeline captures both experimental data for each sample (e.g., gene expression values for 20,000 genes) and sample metadata (e.g., disease, treatment, survival, mutation status) for each sample. Sample metadata is typically provided by authors in unique, study-specific terms which are understandable within the context of that study, but which are not aligned with terms used by other authors, often creating artificial and unnecessary distinctions between similar data. To overcome this difficulty a controlled vocabulary was developed and retrospectively applied to all studies in Oncomine. This step permitted metadata to be organized in an informative, hierarchical ontology, such that users can now find and group similar data appropriately and can easily identify trends across similar data. In addition to these changes to sample metadata, this proposal funded modifications to the database architecture that included implementation of a binary flat file format for the efficient retrieval and storage of gene expression and analysis data, and a relational database format for storing metadata. This fine tuning of the database reduced its size from 100GB to 16GB, and made it possible for Oncomine to not only incorporate more large datasets, but also to conduct re-analyses of the entire database in hours rather than weeks. Finally, the user interface was re-designed to exploit these advances by implementing rich keyword search functionality, exposing the hierarchical metadata structure, developing data summarization tools, implementing persistent IDs that allow users to bookmark any result for immediate recall, and making all visualizations and data available for direct export to PowerPoint or Excel. Importantly, these changes also enabled advances beyond the original scope of the proposal, and have been critical to subsequent advances to the Oncomine platform, such as the addition of DNA copy number data, micro-RNA data, and next-gen sequencing data. Furthermore, our ability to integrate these data types in meaningful ways, and to create visualizations so our users can explore them, is a direct result of this SBIR funding. Overall, funding of this proposal resulted in the transformation of Oncomine from an interesting academic prototype to a stable and scalable cancer genomics database with enormous potential for improving the value of genomics data for commercial drug development efforts. The richness and utility of these efforts is reflected not only in the current implementation of Oncomine, which contains data from 550 studies over 19 major cancer types, but also in our commercial user base, which includes 12 of the top 15 pharmaceutical companies worldwide.

9

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Photoactivated Antimicrobiall Ophthalmic Adhesive

Corneal ocular infections can have a profound and devastating impact on visual function. Ulcerative keratitis is a sight-threatening condition and remains an important cause of blindness that requires skilled management and effective antiinfective treatment to preserve vision. If diagnosis and initiation of

appropriate antimicrobial chemotherapy are delayed, then it has been estimated that only 50% of eyes will heal with good visual outcome. The problem of emerging antimicrobial resistance emphasizes the need to find more effective antimicrobial agents for management of ocular infection.

Purpose: To evaluate the effectiveness of a novel laser-activated collagen-flavin bioglue to treat ocular microbial infections.

Methods: Prior work demonstrated photoactivated collagen-embedded riboflavin-5 phosphate (PCRB) reduces bioburden *in vitro*using both standard and biofilm induction models. This study examined the efficacy in chronic and acute wound models. Female Balb/C mice (n=60, 35g) were anesthetized. Axially oriented 1 cm incisions were made. A cotton swab was inserted in each wound after soaking in 1 McFarland density unit (~3-5 x 10^6 CFU) bacterial suspensions of *Staphylococcus aureus,* MRSA, *Pseudomonas aeruginosa* or *Escherichia coli.* For the chronic model the incisions were closed and after 24 hrs reopened at which time 1 cm x 1 cm collagen/riboflavin-5-phosphate composite wafers were inserted into the wounds and photoradiated. For the acute model the bacterial suspensions were introduced into the wounds 10 min prior to photoradiation. All wounds were irradiated at 457nm (3000mW, 1cm spot diameter) for 15 min using a diode laser (Conversion Energy Enterprises, Spring Valley, NY). Control wounds received neither PCRB or photoradiation. The animals euthanized at 24hr intervals (24 – 144 hr) post therapy. Quantitative wound bacterial counts (CFU/g tissue) were determined using standard microbiological methods to measure bactericidal efficiency.

Results: The results were computed as the percent reduction in bacterial burden relative to controls. PCRB plus photoradiation resulted in statistically significant 2-3 log reduction in bacterial counts($p<0.001$) for all bacterial strains and in both wound models.

Conclusion: Photoradiation at 457nm after PCRB placement inhibits bacterial growth in chronic and acute wound models. The collagen materials used in this study have been proven to be strong ocular adhesives. They are biocompatible and pliable, conforming to any surface configuration and can be used to release the photosensitizer in a desired time period. Planning for clinical trials is in progress.

10

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Therapeutic Ultrasound Contrast Agent Modeling

Target drug and gene delivery are rapidly emerging applications for ultrasound contrast agents since they reduce deleterious side effects to healthy tissue and minimize overall dose needed. Development of new contrast agents requires a good understanding of the effect of the shell materials properties on the dynamics of the agent and on the mechanism of breakup of the microbubble shell. The objective of this SBIR project is to develop an accurate software package to help developers identify and optimize microbubble shell materials. The software helps investigate and understand the effects of shell properties on drug targeting, contrast agent dynamics, and shell breakup in response to directed ultrasound.

The developed software employs advanced numerical models which couple viscous and Inviscid, fluid and structure dynamics codes to allow the user to simulate the dynamics of encapsulated microbubbles under realistic scenarios. These include description of agents- agents and agent- blood cells and/or vessel walls interactions, when excited by ultrasonic waves. The software can accurately capture non-spherical dynamics and shell breakup for interacting and isolated agents.

In the Phase I and Phase II studies we have successfully developed an equivalent finite-thickness shell model to simulate thick-shelled contrast agents and a zero-thickness shell model to simulate thin-shelled contrast agents. The models were first validated against experimental results for an isolated triacetin-shelled bubble insonified by an ultrasound acoustic wave. Non-spherical deformations due to multiple bubble interactions and/or bubble/wall interaction were validated through comparison with laser-generated tandem bubbles and spark-generated bubbles. In addition, the models were applied to the study the mechanisms of cell/tissue damage and sonoporation due to ultrasound-excited bubbles. The deformation of the soft bio-material was shown to modify the bubble jetting.

We are combining our experience in fluid dynamics and material science to develop and offer customers a software package customized for modeling the behavior of contrast agents and drug encapsulated delivery microbubbles in biological systems in presence of an ultrasonic field. Our software will help developers design and select shell materials and practitioners to determine condition for controlled shell breakup in response to directed ultrasound. This will help reduce pharmaceutical efforts required to optimize materials and procedures.

11

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Bioscience Resources for Education

Home schools represent an underserved market that is rapidly expanding in the United States. There is currently a limited number of biomedical and biotechnology based science experiments specifically tailored for middle school students. EDVOTEK, Inc. believes the opportunities provided by these markets are significant and will continue to grow, and it affords a unique business opportunity for the company and is a logical extension of its current business. With the second SBIR from NIH/NCRR (R44 RR021997) the company researched and brought to market 30 “hands-on” pre-biotechnology and life science educational experiments designed to enhance self-directed science learning for the middle schools. The products were tested at the company and in a dozen classrooms in several schools as part of the beta testing. Where appropriate modifications were made before the experiments were submitted to assessment studies on student learning. The assessment studies focused on representative experiments from the three major areas of the life sciences that was the focus of this research.

A second Phase II SBIR grant from NIH/NCRR (R44 RR018670) provided funding to research and develop high school and college PCR-based experiments and thermal cyclers. At this time the company offers over 20 PCR-based experiments and 2 thermal cyclers that are designed to be robust and affordable for educational budgets.

A third Phase II SBIR from NIH/NIAAA (R44 AA015026) provided support for research of experiments that deal with alcohol metabolism. Experiments for which research is completed are based on the enzymology, molecular biology and the effects of alcohol on *C.elegans*.

The experiments from the three Phase II research were supported by an active outreach professional development program for teachers at 15 meetings per year at National and State science education meetings to include NSTA, NABT, ASM. During the support period assessments of student learning were carried out in classroom settings with an IRB oversight issued to the company

Edvotek®, Inc. (www.edvotek.com) manufactures robust research grade biotechnology education experiments, biologics, reagents and equipments for high schools and colleges. Experiments include DNA science, electrophoresis, forensics, PCR, molecular cloning, immunology, environmental science

and A.P. Biology. Products offer hands-on investigations with options for student participation in inquiry-based extensions that bridge science and education. The sister company, DNA Depot®, LLC. (www.dnadepot.com) is dedicated to providing safe, innovative and affordable life science educational resources for students of all ages.

Sample experiments and the two thermal cyclers from all three grants will be displayed.

12

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Laboratory Soft X-Ray Cryo Tomography Microscope

The objective of this project is to demonstrate the feasibility of a cryogenic soft x-ray tomography microscope with a laboratory source. Such an instrument allows 3-dimensional structural imaging of specimens such as whole cells, which can provide crucial missing information to interpret results from functional imaging techniques such as optical fluorescence microscopy. So far, only synchrotron-based soft x-ray microscopes have been generally available to provide such information.

Soft x-ray tomography provides good contrast and penetration of organic specimens without staining, sectioning or other invasive sample preparation techniques, while cryogenic sample handling minimizes the effects of radiation damage during data acquisition.

We present here results from a soft x-ray microscope coupled to a table-top Z-pinch plasma source operating at 430 eV photon energy. The microscope, developed by Xradia Inc., enables tomographic data acquisition at cryogenic temperatures utilizing a cartridge-based sample handling system. It comprises a glass capillary condenser and a Fresnel zone plate to produce images of about 40 nm resolution on a direct x-ray detection CCD camera. The x-ray source is based on the Energetiq EQ-10 platform originally developed for EUV lithography, modified for operation in Nitrogen VI.

Tilt series of Saccharomyces cerevisiae have been acquired, from which a 3-dimensional representation of the specimen has been obtained using tomographic reconstruction algorithms. The results have been further segmented to isolate individual organelles within the cell. Furthermore, a method for correlative imaging of an unstained cell via cryo-fluorescence light microscopy, cryo-X-ray computed tomography and cryotransmission electron microscopy is demonstrated.

While synchrotron based soft x-ray microscopes already exist to obtain such data, this completely laboratory-based approach enables much greater access to biological whole cell tomography revealing a new wealth of three-dimensional volume information without chemical fixation or physical sectioning.

13

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A Simple, Non-Invasive, Effortless and Portable Respiratory Diagnostic Device

We have developed a simple respiratory diagnostic device that can rapidly identify individuals with respiratory disorders, such as asthma, COPD, vocal cord dysfunction, etc. The device is called the Airflow Perturbation Device and works by measuring the breathing pressure and flow and by placing a periodic known resistance in the path of the flow. Once the airflow and pressure are measured the total respiratory resistance of the patient is calculated and immediately displayed on the screen of the device. The device is small, portable and weighs less than one pound.

There are a number of respiratory diagnostic devices used in clinics, the simplest are the spirometers. Spirometers are simple and inexpensive devices, but they are effort dependant, i.e., patient cooperation is necessary and the results are dependent on the degree of effort invested by the individual patient. Our device, in contrast, is completely effortless and the diagnosis is done under normal breathing in less than one minute. This is particularly important for young children.

Respiratory resistance value changes with age and other factors such as height, chest circumference and gender, however, age is the most dominant factor. Respiratory resistance is very high for young children, reduces with increasing age, then remains constant in the adulthood. We have evaluated the respiratory resistance value of more than 2000 individuals 2 to 88 years of age. The values of the respiratory resistance varied from 2 to 8 cmH_2Osec/L

The device can also be useful in monitoring the amount of drug used by the patient. For example, patients with asthma are treated with bronchodilators. The excessive use of bronchodilator may have a negative cardiac side effect. Our device can monitor the amount of bronchodilator administered and warn the patient when no additional drug is necessary. Because of its simplicity and ease of use it can also be used as a home use device.

The NIH SBIR Phase II grant was substantially instrumental in enhancement and further advancement of the device.

14

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2R44CA134063-03; 2R44-AI0717330-05

Advancement of a Novel Cancer Immunotherapeutic into Clinical Trials Through SBIR Granting Mechanisms

Under National Cancer Institutes (NCI) Phase I and Phase II SBIR granting mechanisms we have moved of a novel adenoviral vector platform (Ad5 [E1-, E2b-]) encoding carcinoembryonic antigen (CEA) through pre-clinical evaluation, clinical grade manufacture, obtained a FDA-IND, and now into clinical trials. The Ad5 [E1-, E2b-] vector platform with unique deletions in the E1 and E2b regions can induce potent cell mediated immune and antibody responses against delivered transgene products in the presence of vector immunity, which is a major advancement in vectored gene delivery technologies. The Ad5 [E1-, E2b-]-CE product called ETBX-011 was manufactured using the necessary and sufficient E.C7 manufacturing human cell line under cGMP conditions through a SBIR NCI awarded contract. Our first-in-man Phase I/II clinical trial aimed at evaluating the safety and treatment of patients having over-expressing CEA cancers with ETBX-011 commenced in the fall of 2010 under IND#14325. Currently, we have reached the maximum intolorated dose of $1x10^{11}$ viral particles without any test drug related toxicity including dose-limiting toxicity (DLT). Preliminary analysis of samples from patients treated with ETBX-011 has demonstrated that the immunotherapeutic induced CEA-specific T cell responses in patients as measured by ELISpot analysis. We are advancing the ETBX-011 immunotherapeutic through progressive clinical trials and are exploring other immunotherapeutic applications of the Ad5 [E1-, E2b-] platform under NCI SBIR grants including the treatment of other cancers such as breast cancer.

We have also investigated to use of the Ad5 [E1-, E2b-] platform as a gene delivery system to induce immune responses against infectious diseases such as HIV-1 and H1N1. After encouraging results under a National Institutes of Allergies and Infectious Disease (NIAID) Phase I SBIR grant, we are now conducting non-human primate studies of this potential vaccine under a NIAID SBIR Phase II grant. With this new vector platform we now have the ability to induce disease immunity in the presence of anti-vector immunity (anti-Ad5 immunity), allowing successful immunization and a true "boosting" of immune response against target antigens. Using the NIH-SBIR granting mechanisms we have advanced this promising vector technology thorough pre-clinical studies, human clinical grade manufacture and into clinical trials. We anticipate initiating two additional clinical trials in the near term, one in breast cancer and the other in influenza.

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15

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Molecular Breast Imaging Multi-Institution Research Collaboration

The project entitled "Dual-headed CZT Breast Imager" was a Phase I/II Fast-Track STTR grant that transferred technology developed at the Mayo Clinic to the small business Gamma Medica, Inc (Northridge, CA). The new field of "Molecular Breast Imaging (MBI)" is based upon the use of ^{99m}Tc-sestamibi (a nuclear-imaging contrast agent) to find small breast tumors that are in the early, more treatable stages of progression. The fundamental detector technology for MBI is the semiconductor radiation detector CZT. CZT modules of proprietary electronic design and packaging – together with imaging systems based on assemblies of these modules, are a foundational asset of Gamma Medica. Prior to the funding period, a prototype dual-headed CZT imaging system was configured by Dr. Michael O'Connor of the Mayo Clinic to perform clinical research protocols. The primary aim of this STTR project was to transfer the dual-head imaging format that was built and tested at the Mayo Clinic to Gamma Medica, Inc. for commercialization and beta testing at two additional sites. The product is now available on the market as the LumaGEM™. Software for image reconstruction, display, and analysis was also transferred from Mayo Clinic to Gamma Medica for incorporation into a separate platform that is planned to undergo FDA-approval. The overall research agreement that was negotiated for the STTR resulted in licensing of Mayo Clinic patents and royalty payments on LumaGEM product sales. During Phase II of the project two LumaGEM systems were installed at local sites in the Los Angeles area: Harbor/UCLA, under the direction of Dr. Iraj Khalkhali, and Cedars-Sinai Medical Center, under the direction of Dr. Alan Waxman. Both of these researchers have years of experience in the Molecular Breast Imaging technique. The research imaging protocols at the three sites were approved by each institution's IRB committee, and IRB authorization agreements were executed between Gamma Medica and each of the three sites to fulfill Federal-wide approval (FWA) requirements for the protection of human subjects. In addition to the transfer of the hardware and software technology, a second major goal has been accomplished – that is, the establishment of pilot clinical trials to confirm the initially promising breast cancer detection results at the Mayo Clinic. These pilot clinical trials also include the comparison of MBI to the more established DCE-MRI breast imaging technique. At Cedars-Sinai, a pre-surgical "dual-isotope" protocol has been approved to provide quantitative, dynamic information on tumor uptake to correlate with pathological findings and patient outcome. Currently more than 100 out of a planned cohort of 300 patients have been accrued in the STTR pilot MBI clinical trials. The STTR has met the goals of transferring technology (both hardware and software), establishing a strong research agreement, designing and installing FDA-approved imaging systems, and commencing pilot clinical trials at three research institutions. The LumaGEM product is currently available for sale and ongoing R&D efforts

enhance its competitive performance in the marketplace. An unforeseen additional benefit from the STTR project is the recent transfer from Mayo Clinic of a special collimator and software algorithms that, when combined, lower the radiation dose to the patient by a factor of five.

16

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Streamlined HLA-Typing, in a Microarray Format

Objectives: Current technologies for high-resolution HLA genotyping were originally developed to support organ and marrow transplantation in the clinic. More recently, a need for simplified, high resolution HLA-typing has grown significantly, due to its emerging role as a genetic marker:

To Guide Stem Cell Therapy (marrow, cord blood)
For Companion Diagnostics abacavir and lumiracoxib).
For Automimmune Disease Risk Screening (Type 1 Diabetes, Celiac Disease)

If HLA typing is to be applied to such large scale applications, it needs to be greatly simplified and its cost must be greatly reduced. Therefore we have set out to develop HLA typing technologies that are very simple to use, inexpensive and, ideally, performed on very small samples, with very little up-fr0nt sample processing. In our SBIR, we have invented, validated and now have been funded to seek FDA 510(k) approval of a low-cost microarray approach to HLA typing: optimized so that testing can be performed with very few steps, on less than 10ng of purified DNA, or more importantly, on a microliter of raw blood or raw cheek swab fluid, or raw Oragene™ stabilized saliva: thus eliminating DNA purification & quantitation in the HLA-typing workflow.

Methods Used: Our technology is based on an inexpensive approach to microarray manufacture that we have invented and patented, where unmodified oligonucleotide probes adsorb to an amine-coated surface. Up to 1000 probes are printed per array, and either 12 or 16 separate arrays are printed per standard 1”x3” microarray slide, thus allowing 12 or 16 samples to be processed per slide, in parallel. Either raw samples or purified DNA can be used as the substrate for such analysis, in a simple workflow which entails (starting from DNA or a raw sample) PCR, room temperature hybridization & washing on a lab bench or the open platen of a lab robot, at up to 528 samples in parallel. Hybridization data are analyzed via one of several commercially-available imagers, using software that we have developed, allowing analysis by non-expert users.

Results: We have developed and completed in-house testing of three microarray products, each of which performs complete HLA-A, or HLA-B or HLA-DRB1 typing on a sample. Follow-on products for HLA-C, DQA1 & DQB1 are nearing completion. Good concordance has been obtained in-house for these tests and outside beta testing has been initiated for FDA 510(k) and ASHI certification.

Conclusions: We demonstrate that HLA-typing can be performed in a relatively simple microarray format, with little specialized equipment, other than a slide imager, and with no special knowledge of the complexity of HLA allele structure, on <10ng of purified DNA or on 1uL of blood or 1uL of a raw cheek swab or saliva or a mouthwash sample. We propose that because of its technical simplicity and very modest sample requirements, this simplified HLA microarray technology will enable routine microarray-based HLA typing to become a less expensive component of solid organ transplantation (the focus of the present SBIR) and an enabling component of HLA-typing for companion diagnostics, population scale screening and clinical research.

17

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Electrochemical Technologies for Biomedical Applications at Giner, Inc.

Authors: Linda A. Tempelman, Badawi Dweik, Klearchos Papas (Giner, Inc. and University of Minnesota, Schulzt Diabetes

Giner, Inc., with a specialty in electrochemical technologies, highlights results from three SBIR Phase II projects and technologies in three fields that are at various stages of technology readiness.

Wireless, Low-Maintenance Transdermal Alcohol Sensor. The objective this project funded by NIAAA was to further advance the patented Giner transdermal alcohol sensor technology by producing the next generation hardware and data analysis system. The results of clinical and field studies with subjects consuming ethanol and the noninvasive WrisTAS™ transdermal sensor measuring the transdermal (through the skin) alcohol are shown, including the sensitivity and specificity of the method. The Giner transdermal sensing technology has been incorporated into a commercial product by BI, Inc. for use in monitoring parole/probation/home arrest subjects in the court systems. The next market to be developed is the alcohol treatment patient market.

Field-Deployable Monitor to Assess Personal Exposure to Multiple Heavy Metals. In this project funded by NIEHS as part of the Genes and the Environment initiative, the goal is to measure parts-per-billion levels of four heavy metals in urine samples. The objective is to provide a field tool for epidemiological studies where the environmental exposure to these toxins is needed in analyzing the genes versus environment interaction in prevalence of disease. Detection of cadmium, manganese, lead and arsenic in urine at the requisite levels are shown using an advanced electrode and electrochemical analysis technique. Commercial applications to be developed include a portable, hand-held monitor for field use in human exposure monitoring and also for water quality monitoring.

Portable Gas Perfusion System for Pancreas Preservation. The goal of this NIDDK-funded project is to develop and demonstrate hardware and procedures for preserving the quality of a human pancreas during organ shipment. The preservation method is to continuously provide oxygen, a crucial nutrient, through the vasculature during storage and transportation. We will: 1) describe and show the portable electrochemical oxygen generation hardware; and 2) show the improvement in human pancreatic islet quality by this method compared to the normal storage method for several human pancreata. This method has shown superior organ preservation (with outstanding islet quality) for twenty four hours compared to the eight hour allowable preservation time by the current standard method. The product will be a complete organ preservation system (hardware and preservation protocol), first for the pancreas for islet transplantation procedures and subsequently for other organs such as the kidney and heart.

18

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Kinesia HomeView™ Quantitative Home-Based Monitoring of Parkinson's Disease Motor Symptoms

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The current standard for evaluating motor impairment associated with Parkinson's disease (PD) is the motor subscale of the Unified Parkinson's Disease Rating Scale (UPDRS-III), a qualitative assessment completed during an office visit. However, these single assessments provide only a snapshot of motor impairment and do not adequately reflect symptom fluctuations in response to medications throughout the day. Therefore, our objective was to design, build, and clinically assess Kinesia HomeView™, a home-based system for quantifying PD motor symptoms. Ten subjects with idiopathic PD used Kinesia HomeView at home for five consecutive days. The system includes a wireless finger-worn sensor containing accelerometers and gyroscopes for measuring three-dimensional motion and a touch screen tablet PC that uses video instructions to guide patients through an automated UPDRS-III-based motor assessment. Each assessment, which includes evaluations of rest, postural, and kinetic tremor as well as finger tapping, hand grasps, and pronation-supination, takes approximately five minutes to perform and was repeated five times per day. Timing of medication dosage was recorded using the integrated Kinesia HomeView medication diary. After each automated motor assessment, algorithms developed with previous SBIR funding scored tremor, bradykinesia, hypokinesia, and dysrhythmia on a 0 – 4 severity scale using the recorded kinematic data. At the conclusion of the study, a clinical report was generated detailing symptom severities and medication usage throughout each of the five days. To ensure subjects performed the assessments correctly in the home, compliance criteria were developed from kinematic data recorded from ten non-PD control subjects, who used Kinesia HomeView in a controlled laboratory setting. The compliance criteria were based on the presence of voluntary motion as well as kinematic features specific to each task. Nine of the ten PD subjects successfully donned the motion sensor, followed video instructions, and performed the motor tasks as directed. Using the developed compliance criteria, over 97% of individual assessment tasks were performed correctly. Based on previous clinical evaluations, all PD subjects' motor symptoms were thought to be well controlled; however, across the studied subjects, rest tremor scores fluctuated an average of 1.3+/-0.6, while finger tap speed and amplitude scores fluctuated 0.8+/-0.4 and 1.6+/-0.4, respectively, on the 4-point scale. As demonstrated in the clinical testing, Kinesia HomeView allows for more continuous monitoring, enabling the capture of motor fluctuation occurring throughout the day in relationship to medication cycles. This increased temporal resolution may help clinicians tailor medication regimens and aid in the evaluation of novel treatments.

19

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Systems Biology Platform for Diagnostic Biomarker Discovery

The development of accurate diagnostic biomarker signatures of newborn inborn errors of metabolism (IEMs) is critical for overcoming the technical limitations of analyzing currently screened biomarkers that heavily rely on statistical analyses. The overall goal of our NIH funded Phase II SBIR program is to develop novel diagnostic metabolic signatures for IEMs using GT Life Sciences' proprietary mechanistic-based systems biology approach. The ability of our computational algorithm and metabolic model of

human hepatocyte was previously demonstrated in the Phase I program to retrospectively identify biofluid-based metabolic biomarkers for IEMs with up to 89% agreement in our preliminary analysis.

The first year of the Phase II project focused primarily on refining our computational platform to achieve higher efficiency and functional capabilities. The hepatocyte model was expanded using liver-specific proteomic data from the Human Protein Reference Database (HPRD). The publically available proteomics data was used to support the addition of metabolic enzymes in the reconstructed hepatocyte model. Our model expansion in this first year resulted in a substantially larger hepatocyte model of 1,826 gene transcripts, 2,180 metabolites, 1,944 reactions, a 175% increase in biochemical reaction content. In addition, the hepatocyte model was validated with a comprehensive list of 350 hepatocyte physiological capabilities (e.g. bile acid synthesis, biosynthesis of non-essential amino acids and lipids). Compared with previously published liver metabolic network (Jerby et al. 2010, Gille et al. 2010), our hepatocyte model has been manually curated and reconciled, and it currently represents the most comprehensive, high-quality genome-scale network reconstruction of liver metabolism.

In addition, we improved our computational algorithm to achieve higher efficiency by increasing the computational speed 10-fold compared with our previous implementation. We also expanded the functional capabilities of our platform to evaluate robust biomarkers at: (1) different levels of IEM enzyme deficiency to account for IEM heterozygosity; and (2) varying nutrient supplementation that may have an effect on an IEM biomarker outcome (i.e. increased nutrient uptake may result in false-positive elevation or false-negative masking effect on biomarkers). Hence, our effort to date has resulted in the refinement of a high quality metabolic network reconstruction for the human hepatocyte, as well as the improvement of the biomarker algorithm to increase the efficiency and breadth of the IEM analysis. Experimentally verifying new biomarkers for high false-positive screened IEMs to develop diagnostic signatures will be the critical next step in this Phase II project.

The commercial plan is for GT Life Sciences to exploit the IEM signatures it develops with testing laboratories, equipment providers to those laboratories (such as those offering tandem MS-MS equipment, reagents and software to measure the key metabolites), or diagnostic companies, through licensing arrangements or other types of partnerships. Additionally, the developed platform will be used to pursue other important commercial applications for biomarkers, including the identification and validation of biomarkers for cancer (and resulting products for use as diagnostics, therapy selection and monitoring aids), toxicology and safety testing, and drug discovery.

20

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A Nanotechnology Enabled Protein Screening Platform with Initial Applications in Early Stage Cancer Detection

Objectives: The objective of Inanovate's Phase II SBIR proposal is the development, evaluation and validation of an accurate, low cost diagnostic test that can discriminate prostate cancer from benign prostatic hyperplasia to a Specificity of 85% at a Sensitivity of 85%.

Technological Innovation. Inanovate has identified a series of auto-antibody biomarkers capable of distinguishing prostate cancer from BPH to a Specificity of 85% at a Sensitivity of 85%. The proposed test integrates this panel with a novel biochip platform capable of quantitatively screening multiple low concentration proteins from blood samples at the point of care (PoC). The platform includes a nano-particle surface technology that improves assay sensitivity by controlling the distribution of proteins across the biochip surface; and a real-time biochip scanner and analysis methodology that provides

kinetic information on biomarker interactions, and enables discrimination of 'specific' (true) biomarker signals from 'non-specific' (false) signals.

Methods and Plan: Inanovate's existing platform will be optimized for performance with test assays as well as the identified prostate cancer biomarkers. Additionally, an upgraded automated version suitable for use at the point of care (primary care clinics) will be designed, constructed and demonstrated. We will complete assay optimization and biomarker validation studies on the prostate cancer biomarkers, utilizing the optimized existing real-time platform (developed through Phase I). Once platform and biomarker panel objectives are completed, the integrated diagnostic test will be prepared for multi site clinical trials and FDA approval. These objectives are addressed through three Specific Aims: 1. Development and Demonstration of Automated, Real-Time Screening Platform; 2. Validation of Prostate Cancer Biomarker Panel; 3. Regulatory and Commercial Preparations for Multi-Site Trials.

Aim 1: An optimized laboratory version of the platform will be developed to facilitate biomarker validation work in Aim 2 and allow the determination of performance characteristics for the PoC platform. Additionally, two PoC versions will be developed, tested and prepared for deployment at clinical settings with the validated markers from Aim 2; and also prepared for clinical approval as a 'stand-alone' protein screening platform.

Aim 2: The system from Aim 1 will be deployed at BWH (via a subcontract) to facilitate access to relevant biomarker and clinical expertise alongside access to required clinical samples for optimal completion of the proposed biomarker validation work. Bioinformatics/data analysis will be subcontracted to the Dana Faber Computational Biology group to ensure the generated data is processed efficiently, and the resultant biomarker panel is optimized in preparation for multi-site clinical trials and subsequent FDA approval.

Aim 3: This brings together all prior work and positions the platform, test and associate data for multi-site clinical trials. Regulatory consultants will be used to guide this process until Inanovate's internalizes the relevant expertise.

Results and Conclusions: There are two key results targeted:
1. Validate automated screening platform for sub 5% assay to assay CV's and sub pg/mL limits of detection from patient sera.
2. Validate marker set to 85% specificity at 85% sensitivity for discrimination of prostate cancer from BPH.

Results will be known towards the end of 2012/early 2013.

21

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GeneTegra: Semantic Integration of Biomedical and Genomic Data

The main objective of our project is the development of the GeneTegra system, an information integration environment designed to facilitate concept-based search and querying of genetics and biomedical data from diverse and heterogeneous data sources. GeneTegra utilizes Semantic Web technologies to address the two main obstacles in the integration of knowledge: syntactic heterogeneity, where data sources have different representation and access mechanisms, and semantic variability, where similar lexical terms may refer to multiple concepts or dissimilar terms may refer to the same concept. Development of the system has included methods for generation of ontology models, ontology alignment, query construction, query planning, and query execution. Ontology models of sources capture the semantic information that exists within the structure of the source of data; they can be generated from different formats, including relational databases, XML and RDF data sets, and structured text files.

Ontology classes are created to represent the main concepts found in data sources, such as tables in relational databases, or nodes in XML. Ontology properties are used to represent the attributes of these concepts and their values. An ontology alignment algorithm, named ASMOV, then finds semantic correspondences among these concepts and properties. ASMOV relies on a set of similarity measurements and a process of semantic verification that ensures that semantic coherence is maintained. A graphical user interface permits users to explore the ontology models of different sources, manage the process of ontology alignment, and define ontology-based queries against multiple data sources as if they were a single data environment. The resulting queries are then distributed using an algebraic manipulation mechanism which seeks to find an efficient plan for execution of the query while guaranteeing that the proper results are obtained. The algebra used for this query distribution and planning is based on a set of mathematical properties defined for the SPARQL query language. Execution is performed by translating queries into the native format of each data source, retrieving results from each source, and then joining all results and presenting them to the user. GeneTegra has been tested in multiple scenarios of biomedical data integration. In this poster, we describe its application to a study to find human genes that are targeted by a particular microRNA. Lists of experimental and predicted microRNA target genes are available online but cannot be easily compared against each other as they are stored in different formats and make use of different gene identifiers. GeneTegra was used to integrate five different microRNA data sources with the UCSC genome database. Queries were run to resolve identifiers and obtain a list of microRNA target genes supported by a majority of the data sources. GeneTegra's comprehensive ontology-driven approach facilitated the cross-comparison and interpretation of gene lists with other types of Semantic Web-formatted data and made it possible to distill the knowledge stored in related biomedical data of disparate formats.

22

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Novel Photoreactive Nanofibers

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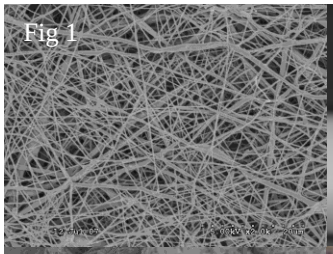
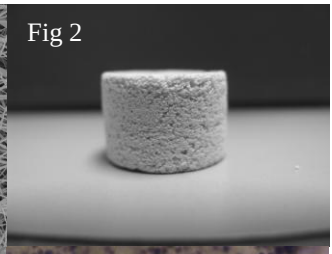
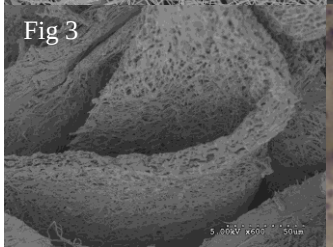
Objective: The goal of this Phase II project is to develop a fully synthetic, biocompatible, biodegradable, mechanically strong and porous bone tissue engineering scaffold filled with nanofibers ready for biomolecule functionalization.

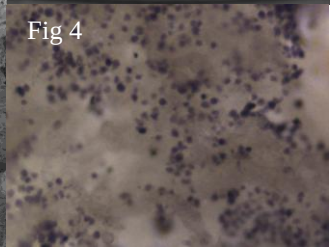
Background: Tissue engineering offers the possibility of aiding regeneration of tissue damaged by disease or trauma and, in some cases, of creating new tissue and replacing failing or malfunctioning organs. It is achieved through the use of degradable biomaterials to induce surrounding tissue and cell ingrowth or to serve as temporary scaffolds for transplanted cells to attach, grow, and maintain their differentiated functions. The role of the scaffold is temporary, but crucial, to the success of the strategy. Therefore, the selection of an appropriate scaffold material is critical. An ideal tissue engineering scaffold is biocompatible, biodegradable, porous, functionalizable and mechanically strong.

Methods: Photoreactive polycaprolactone (PCL) nanofiber meshes were prepared by electrospinning of PCL solutions containing selected amounts of ISurTec® proprietary photocrosslinker, ISurLite™. Nanofiber surfaces were functionalized with polyamines or polyacids using long wavelength UV illumination. Functionalized nanofiber meshes were disintegrated and fabricated into 3D scaffolds with a proprietary sintering process. Bioactive molecules were conjugated to 3D nanofibrillar scaffolds through surface









functional groups. HRP and BMP-2 were used to demonstrate the bioactivity of conjugated biomolecules. C2C12 cells were seeded in the functionalized 3D nanofibrillar scaffolds and cultured in full medium at 37ºC for 3 days.

Results: Figure 1 shows uniform photoreactive nanofibers containing different amounts of photocrosslinker can be co-electrospun. Figure 2 displays 3D nanofibrillar scaffold prepared by sintering, yielding a compressive strength of greater than 0.1 MPa. Figure 3 demonstrates typical microporous and nanofibrillar structures of 3D scaffolds. The amine and carboxylic acid functionalized scaffolds were further modified with HRP or BMP-2 and the conjugated proteins remained bioactive during storage and experimental conditions. Figure 4 confirms successful C2C12 seeding in functionalized 3D nanofibrillar scaffold (nucleus staining).

Conclusion: Natural ECM does far more than just provide a physical support for cells. It also provides a substrate with specific ligands to further support cell adhesion and migration, and regulates cellular proliferation and function by providing various growth factors. Photoreactive nanofibers provide a versatile platform for surface modification and biomolecule decoration of nanofibers, leading to fully synthetic and biologically functional ECM. These photoreactive nanofibers are expected to find wide application in tissue engineering, diagnostics, bioseparation, drug discovery, drug delivery and biocatalysts.

23

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A Novel Combination of Thermo-responsive and Nanofibrillar Surface for Cell Culture

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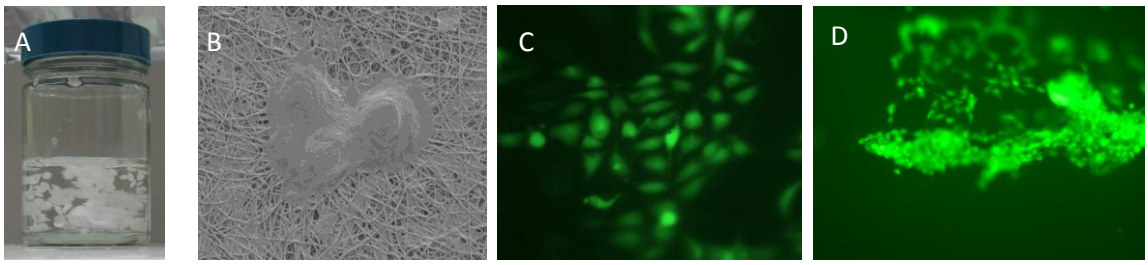
Objective: The goal of this Phase II project is to develop thermo-responsive nanofibers as disc shaped microcarriers for cell culture. Nanofibrillar surfaces, which architecturally mimic the native environment of tissue forming cells, support *in vivo* like growth, morphology and biochemical function of mammalian cells in culture. A thermo-responsive polymer coating on nanofibers allows cells to be released from the substrate by gentle cooling to room temperature, rather than by cumbersome and damaging enzymatic treatments that are currently state-of-the-art for cell detachment. The disc shaped microcarriers provide an anchorage surface per unit volume higher than any spherical shaped competitors. The nanofibrillar thermo-responsive microcarriers will enable high density *in vitro* eukaryotic cell culture under conditions that are minimally damaging and highly physiologically relevant, in contrast to conventional microcarrier systems.

Methods: The nylon nanofibers spun at ISurTec using a 10 syringe pump. Thermo-responsive polymer poly (N-isopropylacrylamide) [PIPAAm] was covalently immobilized on Nylon nanofibers sheets using ISurTec's proprietary crosslinker ISurLite ™.The PIPAAm coated sheets were then fabricated into 3mm disc shaped microcarriers. These microcarriers were characterized based on density measurements, SEM analysis and cell culture studies.

Results: We have successfully synthesized bead free nanofibers with fiber diameter of 200-500nm. The density of discs was found to be close to 1.0 gram/cm^3 and they were readily dispersed in 0.9% NaCl solution by gentle stirring [A]. The electrospinning conditions were adjusted to reduce the pore size to less than 5.0 µm. Reduction in the pore size allowed the cells to attach on the surface of nanofibers rather than penetrating inside the nanofibrillar network [B]. The thermo-responsive behavior of the coated discs was evaluated with the change in culture temperature. At 37°C the substrates are hydrophobic which allowed the cells to attach and proliferate [C]. When the temperature is reduced to room temperature the surface becomes hydrophilic resulting in the release of cells [D].



A) Nylon nanofibers sheets fabricated into 3.0mm microcarrier discs. B) SEM image of Bovine Aortic Endothelial Cells (BAEC) growing on top of Nylon nanofibers C) BAEC growing on nanofibrillar discs stained with calcein to show healthy and robust cell growth D) BAEC detached from PIPAAm coated discs upon cooling to room temperature

Conclusion: The advantages and benefits of ISurTec's nanofibrillar disc microcarriers over standard technologies are

- *In vivo* like growth substrate for optimum cellular function
- High yield of cells per unit volume of culture medium
- Minimum alteration to the viability of cells following detachment from the microcarriers
- High surface-to-volume ratios
- Less susceptibility to shear forces from impeller blades
- Higher mass transfer of nutrients and oxygen

In addition to nanofibers, ISurTec's high performance thermo-responsive coatings have been applied with success to a variety of cell culture surfaces. These include multiwell dishes, flasks, cell factories, roller bottles, spherical polystyrene microcarriers and microporous inserts.

24

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An Advanced, Clinically Practical Laser System for High Precision Surgeries

Objective: The goal of this SBIR project is the development of a laser system for advanced neurosurgeries and other surgical applications requing extreme precision.

Background: Prior work has shown that infrared laser light having certain very specific properties can cut neural tissues efficiently with extremely limited collateral damage; however, previous methods used to generate and suitably deliver such light proved too costly and too complex for widespread clinical use.

Method: We have designed and developed an compact laser system based upon certain solid-state laser technologies and nonlinear frequency conversion techniques. These systems are very robust and reliable, and they can be produced at price levels easily affordable by most hospitals and clinics. The output of these systems was used to ablate various tissues under circumstances that mimic those needed for human surgerical application. Tissue removal rates were measured as was extent of damage to adjacent tissues.

Results: Here, we present results of in-vitro studies made using the advanced surgical laser system we have developed. This system is relatively compact and inexpensive. Using it we have demonstrated extremely clean cuts of neural and other tissues as borne out by microhistologic analysis. We present results of studies showing that collateral tissue damage can be limited to less than 15 µm surrounding the laser incision. Volumetric tissue removal (ablation) rates were in the 2-5 $\times$ 10^{-3} mm^3/s range and can go much higher.

Summary: The implication of this work to date is that tissue more than one or two cellular diameters removed from the incision can be left viable. Moreover, mock surgeries performed to date confirm volumetric tissue removal at rates that are surgically useful. These rates are comparable to those used in prior successful human surgeries for optic nerve sheath fenestration and brain tumor excision. Continuing efforts are focused on further development of the laser and delivery systems for the purposes of increasing cutting speed, improving ergonomics and flexibility, designing a more efficient beam delivery system, and packaging the laser for routine clinical use. We continue to explore its utility for cutiing various tissues and for other possible clinical uses. Planning is in process for eventual human trials.

Conclusion: We have demonstrated the practicality of a new laser tool well adapted for widespread surgical use. The resulting surgical tool is, by its nature, very well adapted for incorporation into robot surgical systems and can be readily supplemented by real-time visual guidance systems.

25

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Potent CDC, ADCC and Anti-Tumor Activity of Human Monoclonal Antibodies to Sialyl-Lewis[a]

Objectives : The long-term objective of our studies is to select a clinical candidate human monoclonal antibody for the treatment of recurring cancers. Monoclonal antibodies recovered from individuals vaccinated with cancer vaccines are characterized to select the most biologically active candidates.

Methods: In this study we utilized the carbohydrate antigen sialyl-Lewis A (sLea), also known as CA19.9, which is widely expressed on epithelial tumors of the gastrointestinal tract, on breast cancer cells, and also on small cell lung cancer cells. sLea serves as a ligand for epithelial leukocyte adhesion molecules and over-expression of sLea appears to be a key event in invasion and metastasis of many tumor cells. Since tumor cells expressing sLea are highly susceptible to antibody mediated lysis mechanisms, sLea presents an attractive molecular target for tumor therapy in a minimal disease setting.

Hybridomas were generated with human B cells isolated from individuals vaccinated with MSKCC's cancer vaccine. Antibody producing cells were identified by ELISA using sLea-HSA and synthetic sLea –PAA-biotin conjugates. Binding to the native antigen expressed on the cell surface was tested by FACS analysis on sLea positive DMS-79 cells and others and the biological activity was measured in CDC and ADCC assays. The binding affinity was determined by Surface Plasmon Resonance (BiaCore) and the specificity of the carbohydrate binding was evaluated by ELISA and by glycan array analysis (CFG, Array 4.1). Recombinant antibodies were generated in CHO cells and purified on Protein A (IgG) or hydroxyapatite (IgM), respectively.

Results: We report the discovery and initial characterization of fully human antibodies that were generated from blood lymphocytes from individuals immunized with sLea –KLH vaccine. Two antibodies were selected for further studies based on the apparent high affinity, which was estimated by BiaCore at 0.14 nM for 5B1 (IgG/λ) and 0.04 nM for 7E3 (IgM/κ). Both antibodies were highly specific for Neu5Acα2-3Galβ1-3(Fucα1-4)GlcNAcβ and did not bind to sialyl-Lewisx, Lewisa, and other related carbohydrates.

Both antibodies have been expressed as fully functional human recombinant antibodies in CHO cells. Complement dependent cytotoxicity (CDC) against DMS-79 cells was approximately 60% and 70-90% for r5B1 and r7E3, respectively. Moreover, r5B1 antibodies showed approximately 50% ADCC of DMS-79 cells with human NK cells (at 5:1 ratio) and 80-90% ADCC with human peripheral blood mononuclear cells with two different blood donors (at 100:1 E/T ratio). These antibodies were tested in two xenograft models: 1) Treatment of animals with 5B1 on the day of engraftment with DMS-79 cells in a subcutaneous model completely prevented tumor growth. 2) Delayed treatment with various doses of 5B1 showed dose dependent protection up to complete cure in SCID mice engrafted (IV) with Colo205 cells. 7E3 antibodies did not show higher protection despite increased apparent affinity.

Conclusion: The specificity and potency of 5B1 antibodies in CDC and ADCC assays translates into good activity in xenograft models. Further studies are warranted to explore the activity against additional cancer specific cell lines in animal models and to pursue preclinical development of 5B1 antibodies.

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26

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ROMP-Derived Oligomers for Facilitated Synthesis

A Phase II Small Business Technology Transfer study between Materia, Inc. and the University of Kansas, which builds upon successes in a STTR Phase I (R41 GM076765) through scale-up of selected ROMP-derived oligomeric reagents and scavengers is reported. This collaborative proposal between the University of Kansas and Materia, Inc. in Pasadena, CA aims to commercialize and expand the current array of functionalized oligomers and optimize the process for their commercialization. More specifically, Phase II has focused on three key areas: (1) Commercialization of Generation-1 (G1) high-load, ROMP-derived oligomeric reagents/scavengers for use in high throughput chemistry, (2) Development, production and application of Generation-2 (G2) high-load, ROMP-derived oligomeric reagents/scavengers and their precursor Nb-tagged monomers and (3) Development and application of norbornenyl (Nb)-tagged G1 monomers for use in high throughput chemistry via capture-ROMP protocols and monomer-on-monomer transformations. Application of these reagents for drug-discovery has been demonstrated through a number of preliminary studies, industrial beta-testers and publications. Through collaboration of scientists at Materia, Inc. and the University of Kansas, success has been achieved in the challenging technical aspects of this Phase II program.

27

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Validation of a 3D Skin Model for Cosmetic, Chemical and Medical Device Phototoxicity Testing (EPARS)

We have enhanced our validated in vitro phototoxicity test using human skin models by exploring inflammatory mediator and gene expression endpoints. The Enhanced Phototoxicity Assay in Reconstituted Skin (EPARS) is based upon a 3D skin model that closely parallels human skin morphology. Major advantages of this test system are that test substances can be applied topically, avoiding the problems of (1) difficulty in solubilizing test materials, and (2) indirect application of test materials to cell monolayers via culture media. In addition, the tissues are composed of differentiated layers of primary human keratinocytes, a more relevant model than mouse tumor fibroblasts. Phototoxic effects are determined by measuring the viability of UV irradiated vs. non-irradiated exposed tissues. In order to increase the sensitivity and specificity of the test, we have measured the release of cytokines into the culture media via ELISA. The release of the inflammatory factor PGE2 was shown to be an early predictor of the toxic effects demonstrated in the viability assay. When compared to human phototoxicity test results and the 3T3 NRU PT validation test material set, EPARS had 100% accuracy, sensitivity and specificity. Microarray analysis of gene expression showed that chlorpromazine treatment with UVA irradiation caused changes in gene expression over time that were not observed without UVA irradiation. These genes include those for keratins, collagens and fibronectins. EPARS is an accurate and sensitive test for detecting phototoxic substances at doses representative of those that cause actual human skin reactions. Thus, EPARS is a highly predictive phototoxicity assay, with endpoints of inflammatory mediator and gene expression that allow for investigation into the mechanisms of photosensitivity in a wide variety of consumer products.

28

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Disrupting Medical Education, One Course at a Time

Study Objectives: The objectives of these SBIR/STTR studies were to develop commercially viable online continuing medical education programs that would improve educational outcomes and meet nationally-recognized physician education needs. It was hoped that, over time, such educational innovations might lead to lower costs and higher quality in health professional education.

Methods Used: We developed four online, case-based, education programs to improve physician recognition and management of skin cancer, domestic violence, cultural conflict, and pain management. As part of this work, we also developed practical educational outcomes assessment tools. All education programs were evidence-based and authored by national experts. All programs were found to be educationally effective in rigorous clinical trials. After testing, all programs were offered for sale via a respected e-commerce Website.

Results: This work has the potential to stimulate a "disruptive innovation" in medical education that could improve quality and reduce costs. There is increasing use of digital technologies in medical education, but, outside of biomechanical simulators, most educational technology innovations have been based on existing educational (synchronous) approaches, such as e-mail distribution of slides and course materials. More innovative work has tended to be based on demonstration of concept, without rigorous evaluation or a commercial model. This work addresses these shortcomings in several ways:

- All online programs fully use the interactive and multimedia capabilities of the Internet.
- All programs are asynchronous, allowing for scalability and mass distribution.
- All online programs have been extensively studied and found to be educationally effective and extremely well-accepted by physicians. The pain management program, for example, was studied in a randomized controlled trial of 95 community physicians. It produced a 3-month improvement in educational outcomes that was significant and comparable to that produced by live expert lectures of similar length.
- These four projects have led to 10 peer-reviewed publications that have been frequently cited by national reviews of online education.
- All online programs have been marketed commercially since their completion. So far these programs have been used by 6,300 physician members of The Virtual Lecture Hall® (www.VLH.com).
- There is increasing interest in adapting these and similar programs for use in formal medical training program. For example, current SBIR work deals with substance abuse management and targets medical residency programs.

Conclusions: There is a well-recognized need within the health professions to lower training costs and improve quality. Experience shows that such improvements typically arise from what Chistensen characterizes as "disruptive innovations". A disruptive innovation usually begins in an underserved population and requires a critical mass of innovation and improvement to progress. This 10 years of SBIR/STTR-funded work has begun to produce such a critical mass of medical education innovation. It started by helping meet the continuing medical education (CME) needs of younger practicing physicians and is now moving up-market to more formal physician training programs.

29

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Quality Control Parameters for Radioactive Gold Nanotherapeutic Agent for Tumor Therapy

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Background: Approximately 192,280 men in the United States are diagnosed with prostate cancer every year killing 28,000 men in the U.S.; and 288,000 worldwide if not treated in time. Therefore, development of new and improved therapeutic modalities, especially for treating inoperable prostate cancers, would be of profound clinical importance.

Objective: The overall objective of our Phase II SBIR project is to develop a new and effective therapeutic modality based on radioactive gold nanotechnology for treating prostate cancer. In this context, Nanoparticle Biochem, Inc (NBI) has developed NBI-29, a proprietary nano-radiopharmaceutical agent. NBI-29 consists of therapeutic radioactive Au-198 nanoparticles surface coated with a FDA

approved glycoprotein. Au-198 provides a desirable beta energy emission and half-life for effective destruction of tumor cells/tissue (max = 0.96 MeV; half-life of 2.7 days). Intratumoral injection of NBI-29 has demonstrated high therapeutic efficacy in suppressing prostate tumor in mice models.

Methods: We have established the following quality control methods for analyzing purity of NBI-29 formulation. NBI-29 is a radioactive nanoparticle and QC parameters should be established for the purity of both radioactivity and nanoparticle. The radionuclidic purity of Au-198 is established using Ge counter.

QC method 1: In this method, the homogeneous distribution of nanoparticles in NBI-29 is ascertained by observing the surface plasmon absorption band and the band width using UV-Visible spectroscopy.

QC method 2: Purity of NBI-29 is established using thin Layer Chromatography. To ascertain complete conversion of $^{198}AuCl_4^-$ to nanoparticles by differentiating Rf values of the starting material and ^{198}AuNPs. Further quality control was established using high performance liquid chromatography using Size Exclusion GE Secrose column was employed in this study by optimizing different eluting protocols to differentiate $AuCl_4^-$ and AuNPs.

QC method 3: Measurement of characteristic nanoparticulate size and charge by Zetasizer

Results: Our studies have established that NBI-29 formulation is highly reproducible. This data is supported by their consistent physic-chemical properties.

Conclusion: We have developed quality control methods for the optimized final formulation to **clinically translate NBI-29 for treating prostate tumor in human patients.** The methods will ensure identity, strength, quality and purity of NBI-29. As the guidelines for evaluating the quality control parameters of nanoparticles for human use are still in the evolutionary phase, we believe our methods can serve as a forerunner for future drugs based on nanotechnology. In the present poster, we will report optimization of quality control parameters for NBI-29.

30

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Biological Response of Dental Plasma Treatment Using Ar and Ar+O_2 Gases

The main objective of this SBIR project is to develop a miniature atmospheric cold plasma brush for dental clinical applications. The innovation is the utilization of the recently developed novel non-thermal atmospheric plasma technology for dental treatment to prevent tooth decay and improve the durability and longevity of dental composite restoration. Non-thermal atmospheric plasma is a gaseous medium that is chemically reactive and has the capability to penetrate irregular cavities and fissures and kill bacteria. Furthermore, plasma species can spread up to a few millimeters into a fluid. This feature make non-thermal plasmas extremely suitable to treat, clean, disinfect tooth decay, and fluorinate enamel in a wet oral environment.

The atmospheric cold plasma was usually generated in argon as a carrier gas and oxygen added as a working gas as needed using a direct current power supply. The cold plasma was then used to treat the dental restoration site (mainly dentin) to enhance adhesion strength at dentin-composite interfaces, actively fight bacterial infections to avoid contamination, and offers additional cleaning to the decayed

matters. The plasma treatment could also effectively fluorinate the tooth surfaces when a fluorine-containing monomer was added into the plasma to prevent caries. The well accepted bench top micro tensile test in dental research area was adapted for evaluation of plasma treatment effect on bonding strength. Plasma disinfection effects were investigated by evaluating the survivability of oral bacteria with plasma exposures.

Our first plasma brush device prototype consisting of a hand piece, a control panel (console) for operation, a power supply, and a gas tank of argon carrier gas and a relatively much smaller oxygen gas tank has been constructed in this Phase II project. The console was designed with user-friendly interface features for start, operation, monitor, control and stop of the plasma brush device in the environment of dental office.

Our recent research results indicate that a stable operation of cold plasma brush at body temperature or less, and oral bacteria destruction by more than 99% in a few seconds have been successfully achieved. In addition, the bonding strength for dental composite restoration induced by plasma treatment of dentin-composite interfaces has been significantly improved.

Although cold plasmas are known to be non-destructive to tissues, cell culture studies using human oral keratinocytes have been performed to investigate the potential side effect of chemical reactive species generated in the plasma torch due to accidental exposure of gum tissue to plasma during operation. Plasma conditions investigated include 10, 30, 60 seconds of plasma treatment using argon alone or argon mixed with oxygen at two power levels of 6 and 10 Watts. The 3 and 7 day cell culture data suggest there are no statistically significant difference between plasma treated and untreated controls.

In summary, the above-discussed results indicate that the dental plasma brush under development at Nanova is a novel dental tool to be used by dentists in dental office for improved dental restoration and dental cleaning without side-effects on healthy gum tissues during its operation inside a patient's mouth.

31

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Low Cost Cell and Tissue Acquisition System

A limiting factor in current research community is its inability to effectively collect particular cell types or specific regions from a heterogeneous tissue or cell culture samples. Tissue microdissection and cell sorting technologies have advanced in the last decade from manual dissection to sophisticated but costly laser capture microdissection (LCM) and high speed fluorescence assisted cell sorting (FACS). The advantage of the proposed cell and tissue acquisition system (CTAS) is its cost effectiveness and ease of use compared to the existing technologies of LCM and FACS.

CTAS is a novel capillary-based vacuum-assisted device that can be attached to an inverted microscope to dissect tissue slices and cell cultures at the cellular resolution. High quality RNA, protein, and DNA are isolated from CTAS dissected samples, suitable for microarrays, Western blots, and sequencing. Further, CTAS may collect cells without affecting their viability so that the collected cells can be used for further culturing. The current prototype has been tested to demonstrate its capabilities with tissue samples from the central nervous system, i.e. brain and spinal cord. This instrument can be applied for the collection of various tissue and cell types, and is capable of collecting subanatomical regions, cell clusters and specific cells from native tissues, fixed or live.

CTAS includes a linear actuator assembly at the top for the movement of the capillary; vacuum module that controls and provides adjustable vacuum strength and duration; mechanical x-y stage for positioning

of the sample in alignment with the capillary tip; and two gooseneck LED illuminators. A disposable filter unit above the capillary prevents contamination of the vacuum path. The disposable capillary component has an easy-to handle needle hub attached, which also makes a sealed connection with the filter unit. Optimal operational parameters have also been estimated.

The user will use the X-Y mechanical stage to locate the tissue/cells of interest and press a button to initiate the suction of those tissue/cells, which will be collected into the capillary. With the trinocular model, the user will be able to operate the device while viewing the computer screen instead of through the binocular lens. The glass capillaries are disposable consumables. The vacuum duration and strength, as well as the calibration for the capillary positioning, are controlled by the box on the right hand side. The X-Y mechanical stage has been placed on the microscope in the opposite orientation to the conventional position to allow the user to access the CTAS buttons and the stage mover more ergonomically. This instrument can be applied for the collection of cells from various tissues, and is able to do so more cost effectively than the existing LCM and FACS.

32

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Novel Enzyme Reagents for Epigenetics Studies

Epigenetic DNA modifications in mammalian genomes, especially methylation, play crucial roles in gene regulation during cell differentiation. Commonly used methods, such as bisulfite sequencing, have many inherent drawbacks. The proposed research in this project aims at providing a set of novel enzymatic reagents for mapping the epigenetic landscape based on a family of newly discovered modification-dependent restriction endonucleases, MspJI enzyme family (Zheng Y, Cohen-Karni D, Xu D, Chin HG, Wilson G, Pradhan S, Roberts RJ. A unique family of Mrr-like modification-dependent restriction endonucleases. Nucleic Acids Research 2010, 38(16):5527-34). We plan to characterize the biochemical properties of these enzymes and develop methods for applying them in epigenetics research. Coupled with high-throughput sequencing technologies, they promise a much simplified pipeline from which myriads of revolutionary methodologies and research can build on.

33

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QDS™-Web, Web-based Survey Data Collection

The objective of developing QDS™-Web, a Web-based data collection system is to provide researchers an easy-to-use, Web-based system that supports complex questionnaire logic necessary when conducting biomedical and behavioral research studies, without requiring software development expertise. QDS™-Web is an extension of NOVA's Questionnaire Development System (QDS™), a complete multi-modal system, including mobile devices, for developing and administering research data collection applications. QDS™ enables researchers to produce all materials needed to administer a questionnaire from a single set of specifications. QDS™ is a modular software system consisting of a Design Studio for designing your survey, multiple data collection modules allowing for varied modes of survey administration, and a Warehouse Manager for data management, meeting clinical trial data

security requirements. QDS™-Web allows researchers to launch new or existing surveys developed within the QDS™ Design Studio on the Internet for Web-based data collection.

QDS-Web was developed using industry-standard Java technologies, including JavaServer Faces, facelets, Enterprise Java Beans, and the Java Persistence API. Java Platform, Enterprise Edition 6 (Java EE 6) was selected as the development platform due to its low cost and wide adoption across the Web, as well as interoperability on both Windows and Unix server platforms. Glassfish 3 was selected as the application server as it was the industry's first applications server to support Java EE6 standards and provides many common services required by an enterprise application. Microsoft SQL Server 2005 was selected as the relational database platform for storing data collected by the QDS™-Web data collection module.

The resulting QDS-Web system consists of two major pieces: (1) application files for administering research surveys and (2) Administrator's tools for launching new surveys, modifying existing surveys, and accessing collected survey data. Survey logic, including questions, information elements, skip patterns, consistency checks, dynamic data substitutions, and calculations are determined at design time by the survey developer, using QDS™ Design Studio. After a survey is designed, the designer/researcher accesses the Web-based Administrator's tool using an assigned username and password to directly upload the new survey into the QDS™-Web system. After specifying some descriptive information about the survey and customizing desired look-and-feel using standard Cascading Stylesheets, the survey is immediately available for administration on the Web. To an end user, typically a survey respondent, the QDS™-Web application appears as a single Web-based survey accessed using their preferred Web browser on a desktop platform (Windows, Mac, Linux) or a mobile platform (iPhone, iPod, iPad, Android). The standard survey interface consists of one question or information element per screen, as well as navigation buttons and a progress bar. Each question in the survey uses standard, 508 compliant HTML form elements (textboxes, radio buttons, checkboxes) so respondents find responding to the questionnaire as easy as a Web-based form on their favorite Web site.

In conclusion, QDS-Web provides researchers with an easy-to-use application for collection of complex research survey data over the Web, requiring no specialized software on the respondent's device, thereby making the survey available to all potential respondents with an Internet connection and Web browser.

34

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Long-term Survival in a Phase II Study of Belagenpumatucel-L (TGF-β Antisense Modified Tumor Cell Vaccine) in Non-small Cell Lung Cancer (NSCLC)

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Using partial funding obtained from SBIR grant R44 CA096025, belagenpumatucel-L **(**Lucanix®), a therapeutic vaccine comprised of 4 TGF-β2 antisense gene-modified allogeneic NSCLC cell lines, was tested in a phase II trial. Seventy-five subjects with NSCLC received monthly intradermal injections of the vaccine. The 2 stage II, 12 stage IIIA, 15 stage IIIB, and 46 stage IV subjects were randomized into three dose cohorts. Median survival for all subjects was 14.5 months and five-year survival was 20%. Stages IIIB/IV subjects enrolled into cohorts 2 and 3 had a median survival of 15.9 months and a five-year survival of 18%. These data compare favorably to historic data in which fewer than 5% of advanced NSCLC patients have five-year survival. For subjects with stable disease or better following frontline

chemotherapy, median survival was 44.4 months and five-year survival was 50%. For subjects who progressed following frontline chemotherapy, median survival was 14.1 months and five-year survival was 9.1%. We performed a number of assays of cellular (ELISPOT and cytoplasmic cytokine expression) and humoral (antibody ELISA) immunity on subjects in the trial and correlated these data with overall survival. Subjects who demonstrated an increase in both cellular and humoral immune reactivity following treatment had a significant survival advantage over subjects who showed an increase in only one measure of immunity with a median survival of 32.5 months vs. 11.6 months ($p = 0.015$), suggesting that both cellular and humoral responses are important to achieve durable clinical benefit. Based on these data, we have instituted an international, randomized, pivotal Phase III trial to evaluate the efficacy of belagenpumatucel-L in a maintenance setting in stage III/IV NSCLC patients who have stable disease or better following frontline chemotherapy. The trial is designed to enroll 506 patients and is powered to measure a 3.5 month survival difference. There are two planned interim analyses. To date, over 228 patients have been enrolled in 49 clinical sites in 8 countries. Confirmation of the phase II data in a randomized, phase III setting would provide an important advance in the treatment of non-small cell lung cancer.

35

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Enabling Non-Invasive Physiological Assessment Systems

Orbital Research Inc. has developed FDA cleared monitoring electrodes that enable reliable and robust physiological data acquisition during extended wear/monitoring applications through the NIH SBIR program. These no-preparation electrodes do not require a gel due to the patented surface features which enhance the performance of the sensors at the skin/sensor interface. These enabling electrodes in combination with ultra-low power advanced electronics are producing family of cardiac monitoring products for extended wear applications. **These extended wear monitoring systems have been targeted to meet the needs of the rapidly growing demand for clinical, outpatient, and elderly health monitoring applications as well as non-invasively monitoring the health of a fetus *in utero*.**

The Orbital Team currently has two primary Physiological Assessment Systems: a non-invasive fetal monitoring garment and an extended wear cardiac monitoring system. The fetal monitoring garment utilizes an array of no-preparation electrodes placed over an expectant mother's abdomen to monitor the fetal ECG waveform. These non-invasively collected ECG waveforms have been demonstrated to be of similar fidelity to ECG waveforms collected from invasive scalp electrodes. Orbital's fetal ECG monitoring system has the potential to reduce the number of unnecessary C-sections which will significantly impact health care costs.

The Orbital Team in partnership with BodyMedia is also developing extended wear cardiac monitoring systems capable of collecting several physiological parameters such as ECG, heart rate, heart rate variability, presence of sweat (galvanic skin response), body temperature and heat flux while also monitoring an individual's motion via a 3-axis accelerometer. Through these data, the Orbital team utilizes BodyMedia's machine learning algorithms to determine the wearer's context or activity of the individual at any given moment – is he/she running, walking, sitting, sleeping, riding in a car, exercising, etc. Thus, we believe this combination of physiologic data will produce a more extensive and accurate understanding of cardiac events and chronic disease status in addition to the effectiveness of therapeutic and pharmacological interventions.

Due to the benefits of the no preparation extended wear electrodes, Orbital's Physiological Assessment Systems will enable researchers and healthcare providers the opportunity to continuously survey physiologic metrics during "in clinic," outpatient, and ambulatory home based (including managed care facility) applications. These Physiologic Assessment Systems will also improve patient compliance because the final product and the individual sensors within the sensor suite are very comfortable and easy to use as compared to traditional sensor monitoring platforms. Compliance is also enhanced due to the product's comfort while being worn for an extended period of time and under free living conditions in everyday settings.

36

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Low Insertion Force Epidural System

Piezo Resonance Innovations, Inc. (PRII) is an ISO 13485:2003, 14971:2007 and Women's Business Enterprise certified medical device development and manufacturing company specializing in the application of science and controlled motion to medical device technology. This Phase II project advances a commercially viable reduction in force tool developed to aid penetrating the ligamentum flavum while also minimizing dural puncture "wet taps" during epidural insertion procedures. In the Phase I NIH SBIR, PRII successfully designed and demonstrated the Low Insertion Force Epidural (LIFE) insertion tool in various tissue models with senior anesthesiologists. In Phase II, the Beta-prototype has been further developed into a viable medical device that is ready for more advanced levels of testing and analysis. Commercial partners have expressed strong interest in taking the LIFE tool to market as part of their product line. In Year II of the project, the device will be tested on human cadaver and porcine models. These developments will lead to a Phase III human study and commercialization in collaboration with a major medical company.

Epidural anesthesia is a form of regional anesthesia involving injection of drugs directly into the epidural space. For obstetrics, a needle is placed within the epidural space through which a catheter is passed to enable anesthesia to be delivered during labor. In the case of pain management, a single shot epidural steroid is often delivered to the epidural space. Local anesthetics are injected causing temporary loss of sensation and pain by blocking the transmission of pain signals through nerves in or near the spinal cord. Needle insertion can be unpleasant to the patient because of the high force levels required to penetrate the supraspinous ligament, interspinous ligament and ligamentum flavum. One of the most common complications is an accidental dural puncture. The epidural space is only millimeters deep. It is difficult to stop the forward momentum of the sharp needle after having pushed through the tough tissue. The dura, or spinal sheath, on the far side of the epidural space, provides comparatively little resistance to the needle. If the dura is punctured accidentally, the chance for a cerebrospinal fluid leak, causing a post dural puncture headache, is quite high. Headaches can be severe and last from weeks to years. Significant leakage can cause enough intracranial hypotension as to tear veins, causing a subdural hematoma, and can cause traction injuries to the cranial nerves resulting in tinnitus, hearing loss, dizziness, facial droop, or double vision. The LIFE device reduces insertion force and enables the clinician a more controlled entry into the epidural space, eliminating the over-shooting that often punctures the dura. The device will utilize an innovative sharps motion control technology with a lightweight, low-cost active device.

PRII has already actively engaged a potential strategic partner that is interested in exclusive manufacturing and sales/marketing/ distribution licensing. In addition, PRII is actively interacting with several Venture Capital and Angel investors.

37

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Development of the On-Demand Semi-Automated Methods for Extraction of Intact Mitochondria from Solid Tissue

Defects in mitochondrial function have been linked to many diseases such as stroke, heart disease, cancer, Type II diabetes, and Parkinson's disease. High quality mitochondria-enriched preparations are needed for proteomic and metabolomic studies. It is believed that such preparations can provide crucial insight into tissue-specific mitochondrial function and dysfunction, and help answer fundamental questions of cell energetics and oxidative stress.

Mitochondria extractions from whole tissue samples are typically performed using Potter-Elvehjem homogenizers or similar labor-intensive, manual disruption methods that require extensive operator experience, often damage fragile organelles, and can result in high sample-to-sample variability. The main objective of this SBIR project was to develop a semi-automated method to enable on-demand isolation of active mitochondria from cells and tissues for target discovery, in-vitro screening of drugs targeting mitochondria, and studies of drug-induced mitochondrial toxicity. Additionally, the high quality subcellular fractions enriched in intact mitochondria are thought to be important for proteomic, lipidomic and metabolomic investigations of mitochondria-associated disorders.

In the course of the Phase II project, we have successfully developed methods for isolation of intact and functional mitochondria from kidney, skeletal muscle, and lung tissue using a novel motorized mechanical homogenizer and specialized hydrostatic pressure instrumentation. These novel methods of tissue homogenization are conducted in individually sealed sample containers under controlled thermodynamic conditions (time, temperature, and pressure) leading to a safer, less cumbersome process and more reproducible results. The quality of mitochondria preparations was characterized by electron microscopy, two-dimensional electrophoresis, and mitochondrial respiration assays. Our data demonstrate that mitochondria extracted by the new methods are intact, functional, and exhibit a protein profile comparable to control samples isolated using a conventional Potter-Elvehjem homogenizer. The resulting mitochondria-enriched samples were also subjected to trypsin digestion followed by nanoLC-MS/MS analysis on the LTQ-Orbitrap. Proteomic profiles of mitochondria samples prepared using the novel extraction methods were found equivalent to those extracted using a standard manual method; they also demonstrated equivalent purity of mitochondrial preparations. As a result of the work funded by the NIGMS SBIR grant, Pressure BioSciences launched two mitochondrial isolation kits (rat lung and rat kidney) in early 2011 and is planning to release two more kits by the end of the year. Pressure BioSciences is currently negotiating with several potential partners to establish business relationships that would enable mitochondrial target discovery/validation services based on the sample preparation methods described above.

38

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Identification of MuRF1 Inhibitors Using a Novel E3 Ligase Assay

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To identify new therapeutic strategies to treat muscle atrophy, we propose to target the ubiquitin E3 ligase MuRF1 for inhibition. Muscle atrophy (wasting), also known as myopathy, is a pathological condition of many diseases, including cancer, AIDS, and diabetes. Myopathy has also been shown to be a dose-limiting side effect of synthetic glucocorticoid treatment, and is a natural consequence of inactivity and aging. Muscle atrophy follows a disturbance in protein homeostasis in muscle, reflected by increased rates of protein catabolism and decreased anabolic activity. The expression of a group of novel genes called atrogins (atrophy-specific genes) has been implicated in the degradation of key muscle proteins. One of these, MuRF1 (Muscle-specific RING Finger), is a RING finger domain E3 ubiquitin ligase and is upregulated in at least 13 different models of atrophy. MuRF1 is associated with a number of myofibrillar proteins and is a very attractive target for preventing or reversing muscle wasting associated with various pathologies. Importantly, MuRF1 knockout mice have been shown to be resistant to skeletal muscle atrophy under starvation conditions and also post-denervation.

To identify novel small molecule modulators of MuRF1 activity, we developed a HTS-assay to screen for inhibitors of E3 ligases. This assay format has been validated for use with a number of E3s, including MuRF1. Utilizing this assay platform, we identified P013222 as an inhibitor of MuRF1. Follow-up experiments confirmed the ability of P013222 to inhibit ubiquitylation of the MuRF1 substrate MyHC in a dose dependent manner and it is now being investigated in cell based models of muscle catabolism. The assay along with our recent progress on the P013222 compound series will be presented.

39

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Fast Quantum-Based Molecular Modeling

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We report recent progress on the development and implementation of new algorithms for DFT (density functional theory). DFT is the most-widely applied quantum mechanical method for modeling chemical and biological reactivity in pharmaceutical industry. For the calculation of exchange-correlation , we have significant progress on a new method called multiresolution exchange-correlation (mrXC) developed recently by our group [1]. MrXC takes advantage of the variation in resolution among the Gaussian basis functions, and shifts the calculation associated with low-resolution (smooth) basis function pairs to an even-spaced cubic grid. We have recently implemented it for GGA functionals with the robust B-spline interpolation. It speeds up the calculation by up to 10 times with no loss of accuracy. For the calculation of Coulomb interaction, we have developed an Ewald-like scheme with Gaussian basis functions. It treats the long-range part of the potential of a compact Gaussian in the reciprocal space and we will show that it is computationally more efficient than the fast multipole method for molecular

calculations. We believe that its combination with our previous development [2] yields the fastest Coulomb method for all electron calculations with full accuracy for molecules.

1. Kong, J., S. T. Brown, L. Fusti-Molnar, Journal of Chemical Physics **124**, 094109 (2006).
2. Fusti-Molnar, L., J. Kong, Journal of Chemical Physics **122**, 74108 (2005).

40

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VISION and NeuroVisions: Innovative E-Laboratories for Science Education

This poster will describe innovative e-laboratories being developed by two Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) funded projects for secondary school and undergraduate science education. The goal of these projects is to create, evaluate, and commercialize elearning materials that allow students to experience, first-hand, the technologies and methods employed by biomedical imaging scientists conducting cutting-edge research. The *NeuroVisions: Teaching Neuroscience with Neuroimaging Data* project (2R44MH070250-02) is creating six e-laboratories for undergraduate instruction based on current research of leading neuroscientists: "Seeing GABAA Receptors at Work: Quantifying Radioligand Binding and its Modulation by Endogenous Signaling Molecules;" “Form and Function: Using Confocal Images of Neuron Structure to Learn About Principal Functions of Individual Nerve Cells;" “Kiss and Run and Other Models of Neurotransmitter Release: Using Advanced 2-Photon Imaging in Mice to Witness Fundamental Components of Nervous System Function;" “Your Brain Without Sleep: Analyzing How Sleep Deprivation Impacts Brain Function;" “Teen Brains on Drugs and Alcohol: Using fMRI to Assess Cognitive Damage and Predict Future Substance Use;" “New Neurons for You: Verifying and Investigating Adult Neurogenesis with Imaging." The Volumetric Imaging for Science Instruction of an Open Nature (VISION) project (2R42HD049973-02) is creating five e-Learning laboratories for high school general biology education: "Exploring Neuron Form and Function: Using 3D Imaging of the Second Brain to Predict Neuronal Shape;" "Stomates Revealed: Investigating How Guard Cells Maintain Homeostasis;" "Surgery Practice: Digitally Defining, Diagnosing, and Excising Brain Tumors;" "How Alcohol Alters the Brain: Exploring Hippocampal Volume Reduction in teenagers Who Drink;" "Mapping the Yeast Cytoskeleton: Reconstructing and Analyzing the Movement of Spindles and Centromeres During Mitosis." The proposed e-labs are being developed on Science Approach’s Moodle-based e-Learning site. For *NeuroVisions*, Science Approach has enhanced the Moodle learning management system with a scientific image analysis tool based on ImageJ and a statistical analysis and graphing interface based on R. Image analysis applications are being developed for the *VISION* project by the New Media Research Institute and the Mayo Institute’s Biomedical Imaging Resource. Formative and summative evaluation of the e-laboratories has been conducted by the West Texas Office of Evaluation and Research. The evaluation has included review by pedagogical experts and faculty, testing by faculty, and classroom testing by students. Evaluation research conducted to date for the *NeuroVisions* project indicates that undergraduate students can easily manage online data collection and statistical analysis functions and particularly enjoy the "apprenticeship" experiences with research technologies and data provided by the laboratories.
Testing of a VISION project e-lab that includes an online tutorial and downloadable computer app indicates strong support by high school science teachers for the usability and affordability of its hybrid elearning model. Science Approach has concluded that its inquiry based e-laboratory format is practical and effective for science education at the targeted grade levels. Plans for commercializing the e-laboratories with the Carolina Biological Supply Company are described.

41

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Improving Activity of SQ641, a Capuramycin analog, for Therapy of Tuberculosis

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Sequella, Inc., a Rockville, MD, pharmaceutical company, in-licensed rights from Daiichi Sankyo (Japan) to develop novel nucleoside antibiotics based on the structure and activity of Capuramycin, a natural antibiotic targeting an essential enzyme involved in bacterial cell-wall synthesis. **Using SBIR Phase I support,** we selected SQ641, a semi-synthetic analog, as best-in-class for activity against *Mycobacterium tuberculosis* (MTB). SQ641 is bactericidal, fast acting, and has prolonged post-antibiotic effect. It synergistically enhances activity of ethambutol (EMB) and has a low frequency of resistance development. It is also remarkably specific for mycobacteria. Despite its extraordinary activity, SQ641 has properties we need to enhance before we advance the drug into preclinical safety, pharmacology, and toxicology studies: low water solubility, poor oral absorption, and inadequate activity against intracellular bacteria. To be useful in TB SQ641 should kill both extracellular and intracellular MTB; to pair with first-line TB drugs, all delivered orally, it would be useful if SQ641 could also be delivered orally. However, intravenous (i.v.) delivery would be appropriate for multi-drug resistant (MDR)-TB (resistance to isoniazid [INH] and rifampin [RIF]), since several of the second-line drugs for this indication are i.v. drugs.

We addressed these issues in a SBIR Phase II grant by:

- *Chemical modification:* We chemically modified SQ641, and we measured changes in solubility, *in vitro* activity against MTB, and both intracellular and *in vivo* activity against MTB. Of the several new compounds derived from SQ641 structure, 2 had improved intracellular antimicrobial activity: SQ641-aua and SQ641-2aua. Interestingly, these compounds also had broader spectrum of activity, and could kill clinically important Gram-positive bacteria.
- *Formulation:* Phospholipid (Phosal 53 MCT) nanoemulsion (NE) formulation of SQ641, SQ641-aua, or SQ641-2aua enhanced killing of intracellular MTB better than any other formulation we tried. Importantly, intracellular activity of SQ641-auaNE/SQ641-2auaNE at 4µg/ml (2xMIC) was markedly better than first-line TB drugs INH, RIF and EMB at 4xMIC. NE formulations of both SQ641-2aua and SQ641-aua enhanced intracellular killing activity of the other TB drugs against MTB. No formulation, however, resulted in oral activity *in vivo* in mouse models of TB infection.
- *Drug activity in vivo.* SQ641 NE formulations were administered to MTB-infected mice i.v. at 25 to 100 mg/kg 2x/wk for 4 wk. SQ641-auaNE was the most efficacious: at 100 mg/kg it reduced MTB colony-forming units (CFU) by 1.5 $\log_{10}$ (lung) and 2.3 $\log_{10}$ (spleen) after only 8 doses. INH, which cannot be used for MDR-TB, delivered orally 5x/wk for 4 wk (20 doses) at 25 mg/kg reduced CFU 3.2 $\log_{10}$ (lung) and 2.7 $\log_{10}$ (spleen).

In conclusion, we identified an injectable SQ641-auaNE formulation that can kill both intracellular and extracellular MTB *in vitro* and has *in vivo* activity.

Sequella, Inc. is looking for a partner to assist in developing a fully synthetic, commercially-scalable process and to synthesize sufficient API to support additional nonclinical studies and preclinical safety analysis.

42

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Micro-patterned Surfaces for Reducing the Risk of Catheter-associated Urinary Tract Infection

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Objective: The aim was to prove *in vitro* the efficacy of a novel biomimetic micro-pattern surface technology (Sharklet™) to inhibit bacterial colonization without the use of antimicrobial agents for use on a Foley catheter to ultimately prevent catheter-associated urinary tract infection (CAUTI), and to demonstrate the feasibility of applying the micro-pattern to the surfaces of catheter tubes.

Materials and Methods: Colonization Assay: *In vitro* testing was carried out against a uropathogenic *Escherichia coli* in tryptic soy broth or artificial urine. Coupons with and without the Sharklet pattern were fabricated in silicone. ~10^6 CFU/ml *E. coli* in growth media was added to Petri dishes containing Sharklet-patterned and smooth control coupons, with ten replicates per surface type. Dishes were incubated at 37˚C. After 24 hours, three days, or seven days, dishes were removed for coupon analysis. Bacteria attached to surfaces were enumerated by sonicating surfaces and plating the cell suspension and visualized via scanning electron microscopy (SEM); micrographs of coupons were analyzed for bacterial area coverage.

Migration Assay: Sharklet-patterned and smooth silicone tube segments were tested for migration of bacteria along the external tube surface using an *in vitro* migration model. Tube segments were cut to 1-cm length and placed tightly between two agar islands in a Petri dish. Agar adjacent to each segment was inoculated with ~10^7 CFU bacteria (either *E. coli* or *Serratia marcescens*). Plates were incubated for 24 hours, at which time the agar on the far side of each tube segment was observed for growth of colonies, indicating migration of the organism over the tube segment. Incidence of migration was recorded for each surface type.

Manufacturing Feasibility: Prototypes of Sharklet-patterned tubes were made using a 3D replica-molding process. Uncured silicone was cast into ar polycarbonate die with size 16 French (5.3mm outer-diameter) shafts. The shafts included a thin, flexible Sharklet-patterned film on the shaft wall. A metal rod with the Sharklet-patterned film wrapped onto the rod was then inserted into the middle of the shaft. The silicone cured overnight and the die disassembled the following day, removing film molds from the tubes, which were then dissected for SEM analysis.

Results: Reduction in *E. coli* colonization ranged from 38% to 58% on Sharklet-patterned coupons and reduction in migration of *E. coli* and *S. marcescens* ranging from 78% to 92% on Sharklet-patterned tubes. Tube prototypes made using the replica-molding process showed good pattern quality based on micro-pattern measurements, which were within 5% of pattern specifications.

Conclusions: The Sharklet pattern demonstrated the ability to inhibit colonization and migration of bacteria through the use of physical surface modification alone. Manufacturing feasibility was demonstrated with tube prototypes exhibiting the pattern on inner and outer tube surfaces. These results suggest that Foley catheters with the Sharklet pattern may prevent bacterial colonization, with

implications for reduced incidence of CAUTI. Next steps include further *in vitro* testing using a more complex migration model and a wider array of uropathogenic species, an *in vivo* 20-patient pilot clinical study, and manufacturing scale-up with an industry partner.

43

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Modulators of LDL Metabolism

Heart disease is the leading cause of death for both men and women in the US, accounting for nearly 40% of all annual deaths. A high cholesterol level is well-known risk factors for heart disease. Although blood cholesterol can be lowered using a number of marketed drugs, of which statins are the leading drugs, only 38% of patients taking these drugs are achieving the low-density lipoprotein cholesterol goals set by the National Cholesterol Education Program (NCEP). Furthermore, patients with homozygous familial hypercholesterolemia who have markedly elevated cholesterol levels respond poorly to current drug therapy, and are at very high risk of premature cardiovascular disease. These and other patients will dramatically benefit from an aggressive treatment of hypercholesterolemia. The long-term goal of our research is to develop novel drugs for cholesterol lowering. Our therapeutic target is the protease proprotein convertase subtilisin-like kexin type 9 (PCSK9). PCSK9 controls the degradation of the low density lipoprotein receptor (LDLR) in the liver and thereby contributes to cholesterol homeostasis. PCSK9 is synthesized as a precursor protein that undergoes processing between the prodomain and catalytic domain. This processing is required for PCSK9 to be secreted and to undertake its biological activity. Our goal is to identify compounds that interfere with PCSK9 function, either by: a) preventing the processing of PCSK9, thus prevent its secretion and its ability to participate in the degradation of the LDLR, and/or b) antagonizing the interaction of PCSK9 with the LDLR. To achieve our goal, we have integrated virtual screening methods and cell-based assays into a simple, efficient procedure to identify hit compounds that can ultimately be optimized to produce a drug for the treatment of hypercholesterolemia. We have developed a customized, robust virtual screening method. We also succeeded in developing and validating a cell-based recombinant assay for screening our compounds. Using virtual screening methods, we screened approximately 1,000,000 diverse, “drug-like” compounds, selected from over 5 million purchasable molecules. After visual inspection of several hundred of the top scoring compounds docked in PCSK9 crystal structures, we selected, purchased and received several hundred screening compounds. These compounds were tested in our biological assays. Several compounds consistently exhibited concentration dependent activity. As part of our SBIR Phase II proposal, we have developed SAR around some of our screening hits, initiated lead optimization, and confirmed the ability of selected compounds to stabilize the LDLR and decrease the low density lipoprotein-cholesterol levels using *in situ* and *in vivo* studies.

44

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Simulation-Based Wound Closure Training System

Objective: The purpose of this SBIR project is to address the need for simulation-based training of open incision surgical skills.

Methods: This surgical skills trainer uses physics-based simulation in an immersive desktop-type environment. The underlying technical tradeoffs favor simulation correctness over interactivity, since we rely on continued growth of computational and graphics capabilities for the same or lesser future cost. We constructed a quantitative evaluation rig that allowed us to use surgical tools to manipulate inanimate as well as appropriately prepared in vitro animal tissue while measuring tissue surface deformation and the loads between the tool and tissue. We used the forceps to grasp the tissue surface and perform movements perpendicular and parallel to the tissue surface, and measured the tissue surface shape at the end of the displacement simultaneously with recording the applied loads.

Results: Our second-generation system is an immersive OR table interface using a consumer-class 120Hz stereo display with active shutter glasses combined with a two-handed haptic interface device configuration developed by modifying the mass market Novint Falcon to provide force and torque feedback and tracking of surgical tool jaws, which provides closure feedback and measurement of strength of grasp (7DOF). We modeled a forearm consisting of multi-layer skin, fascia, muscle, nerves, blood vessels, and bone. We provided needle driver, forceps, and sutures that allow learners to manipulate the wound for closure.

After performing numerical simulation prototyping using commercial and academic algorithms, we developed additional finite element codes specifically adapted for tool-tissue interactions and running on the CPUs/GPUs. We developed an accurate, flexible simulation of suture thread interaction with tools and tissue, incorporating both known and novel mechanics/numerics techniques and a continuous collision detection and response system so that we can provide complex real-time behaviors such as thread self-collision and needle and thread passage through tissue. We ultimately developed our own approach that supports continuous collision detection combined with force and linear complementarity geometric constraints which can resolve hundreds of simultaneous contacts of many types, including constraints that conflict with one another, e.g. pressing a suture into the tissue surface with a tool. We provide force/torque feedback from the tools interacting with the simulated surgical scene through a multi-rate approach that provides effective feel independent of the varying computational load on the multiple processor cores. We send this information to haptic devices that provide simultaneous two-handed 7DOF interaction. We enhanced the Open Scene Graph real-time rendering code and display results on an immersive stereo display.
The simulator can extract metrics for objective assessment of proficiency, such as geometric measures of tool-to-tissue activity and kinetic, force-based monitoring of interaction.

Conclusion: The Open Incision Surgery Simulator combines NIH-funded technology, content expertise of surgical educators, and learning technologies to present material in a challenging, relevant, and compelling fashion. The system described here has been developed to the point of assessing its transfer of training for an initial scenario and plans for expansion of this content and further development of the system to be a commercially robust platform are underway.

45

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Use of the Stoelting OroFacial Pain Assessment Device (OPAD) for Characterizing Neurogenic Inflammatory Pain and Analgesia

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Objectives of Study: We evaluated the effects of pain and analgesics on operant orofacial behavioral outcomes using the Stoelting OroFacial Pain Assessment Device (OPAD).

Methods: The Stoelting OroFacial Pain Assessment Device (OPAD) provides an automated measurement of hot or cold orofacial pain assessment in rats and mice. Using an operant behavior reward conflict paradigm, rodents voluntarily decide between receiving a reward or escaping an aversive stimulus. This is achieved when the animals touches and presses its cheeks between two peltier-controlled temperature elements to obtain a liquid reward. As the temperature (hot or cold) becomes aversive, the animal can withdraw. Using the ANY-maze OroFacial Software, the number of licks, contact with elements and amount of liquid consumed are automatically recorded. The advantage of this system is that it provides investigator-independent pain outcome measures in both rats and mice.

All rodents (male, SKH1-Hrhr mice and hairless Sprague Dawley rats) had *ad libitum* access to food and water between testing sessions, and their weights were monitored weekly. Animals were food fasted for 16h prior to each testing session and trained to drink sweetened condensed milk while making facial contact with a thermal probe, set at 37 °C over a 2-week baseline training period. Rats were then tested at either 37 or 48 °C and two behavioral outcome measures were recorded for each 20min testing session: licks (number of contacts with the sipper tube, which cannot be made without contacting the thermal probe) and stimulus contacts (number of contacts with the thermal probe) which were used to calculate the success ratio (licks/stimulus contact). Neurogenic inflammatory pain was induced using a topical capsaicin (0.035%, 5') cream 30 min prior to testing. In a subset of animals, morphine (0.5 mg/kg, s.c.) was administered concurrently with the capsaicin-treatment.

Results: Hairless *SKH1-Hrhr* mice treated with capsaicin had significantly lower reward licking events (*$p<0.05$) as compared to baseline. Morphine (0.5mg/kg) given concurrently with the capsaicin-treatment provided significant analgesia, as the reward-licking counts were significantly higher than the capsaicin-alone group. Naïve animals or animals given morphine (0.5 mg/kg) 30 min prior had similar reward licking events when tested at 37 °C. Rats treated with capsaicin had a lower themal threshold to terminating the task, indicating development of thermal allodynia.

Conclusion: Capsaicin produced heat hyperalgesia and allodynia, as demonstrated by a significant decrease in reward-licking events and lowering of the task threshold. We evaluated the effects of morphine on capsaicin-induced pain and found that there was an anti-hyperalgesic effect. Note that animals treated with morphine alone did not have a significant increase in reward-licking events as compared to naïve animals when tested at 37 °C. These results suggest that the morphine is providing an anti-hyperalgesic effect rather than simply increasing the rewarding properties of the milk or changing the feeding behavior. The OPAD product represents a significant advancement in pain and analgesia testing since this will be one of the first instruments with high-throughput ability to analyze pain response in rats and mice.

46

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Catalysts for CO Protection in Respirators

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First responders are the men and women who are first on the scene of a natural or manmade disaster and include police officers, firefighters, and emergency medical personnel. This workforce is very large; estimated

to be 11 million state and local first responders in 87,000 jurisdictions throughout the U.S. Escape hoods that are effective against chemical, biological, radiological and nuclear (CBRN) agents need to be developed (and approved by the NIOSH) so that first responders may use them effectively. Current respirators can remove essentially all of the hazardous contaminants except for carbon monoxide (CO) because it is not adsorbed by materials such as activated carbon, zeolites, silica-aluminas and other sorbents. Therefore, the only reliable way to remove CO is to oxidize it to CO_2 which in a respirator requires a catalyst that can operate at temperatures encountered by first responders and other personnel. In addition to the need to protect first responders from carbon monoxide, each year, an estimated 80,000 firefighters battle wildfires. Wildfire smoke contains a large number of toxic components, including pulmonary irritants (particulates) and high levels of CO and as the case with CBRN, there are no suitable respirators available to protect fire fighters from exposure to CO.

TDA Research, Inc. (TDA) has developed several proprietary catalysts that can be added to current respirators to protect the wearer from exposure to carbon monoxide for both the CBRN escape hood and wildland firefighter applications. The CBRN requirement is that the catalyst be tested at 0°C with 64 liters/min of air containing 3600 ppm of CO, with no concentration spike above 500 ppm over 15 min. The wildland firefighter standard is *NFPA 1984 Standard on Respirators for Wildland Fire Fighting Operations*, which requires a challenge of 200 ppm CO at 25°C, 64 liter/min, 92% relative humidity, with a catalyst lifetime of 8 hours. The performance of TDA's catalysts greatly exceeds these minimum requirements, and further, we can tailor the catalyst formulation to meet specific requirements. Our objective is to work with respirator manufacturers to integrate our catalysts into prototypes of their products for further testing.

47

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Power Source Development for Compact Proton Accelerators

Proton therapy is considered the most advanced form of radiation available for cancer treatment, but the size (hundreds of tons with a 90,000 square foot footprint) and cost ($200 million to build) of current proton therapy devices have severely limited the technology's use. For this project, TPL is teaming with Compact Particle Accelerator Corporation (CPAC) and Lawrence Livermore National Laboratory (LLNL) to demonstrate the potential for using TPL's enabling technology to achieve an order of magnitude size reduction and cost reduction. As envisioned, the compact proton therapy devices will be capable of replacing current photon based radiation therapy systems and enable widespread use.

The key to developing this next generation proton therapy device is an extremely compact accelerator design based on TPL's novel, high voltage dielectric. At its current stage of development, TPL's proprietary nanocomposite provides an order of magnitude greater energy storage capability per unit volume when compared to other available dielectric materials. LLNL and CPAC consider TPL's nanocomposite as critical to successfully fulfilling the compact accelerator design. Pulse forming lines are integrated into a single power source to provide the unique combination of energy storage and high voltage proton accelerating voltage. The ongoing NIH Phase II program is in alignment and on schedule with the overall system development.

It is the objective of the current program to demonstrate feasibility for producing a system-level prototype power module that enables the accelerator concept. The two year program includes four stages. Stages 1 and 2 in the first year were focused on establishing the fundamental capability of the cast components required for the accelerator. Stages 3 and 4 in the second year will focus on demonstrating the power module capability and system integration.

Significant progress was made during the first program year. The development is on schedule and on budget. TPL's nanocomposite material and component technology has been demonstrated the voltage

stress capability to meet CPAC's accelerator design targets. Reliable and cost effective methods for casting the pulse forming lines have been established. Individual and stacked pulse forming lines have been fabricated and characterized to high voltage pulse conditions consistent with system requirements. Most recent results from CPAC's testing of TPL pulse forming lines support an operating voltage stress capability of >100 MV/m. (This performance compares with conventional dielectrics with a voltage stress less than 30 MV/m.) Testing is now being performed to establish statistical performance characteristics for operational life. The reliability data from these tests will be used to guide design refinements and initiate scaled manufacturing in the second program year.

The primary objective in the second program year is to scale manufacturing of the power modules and provide components for CPAC's system demonstration. Demonstration of the requisite system-level performance and production capability is scheduled for June of 2012.

48

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Using Interactive Voice Response (IVR) Technology to Improve Hepatitis B Vaccination Compliance in the Korean Community

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Background and Objectives: Limited English Proficiency (LEP) status contributes to the receipt of lower-quality healthcare among patients, including increased likelihood of misdiagnosis and incorrect treatment. Though numerous studies have shown that health-related Interactive Voice Response (IVR) messages can positively influence health status and patient-provider communication, the majority of IVR systems target English-speaking patients. We are designing an innovative interactive-voice response platform that will allow providers to deliver outreach campaigns thereby bridging communication gaps for patients and enabling healthcare organizations to improve quality of care. To determine the effectiveness of this messaging system with patients, we evaluated an educational telephone outreach campaign for Hepatitis B (HBV) with a Korean community in New York City. Specifically, we assessed the effect of campaign messages on compliance with the HBV three-vaccine series, as well as disease knowledge and awareness.

Methodology: A set of culturally appropriate audio messages were crafted, translated, and audio-recorded in Korean. A sample of 110 LEP Korean participants (ages 18–55) were recruited by our community partner and randomized into either the intervention or control arm of a prospective cohort study. The intervention arm received the HBV messages, while the control arm received usual care. Message dissemination followed the course of the vaccine series over a four-month period. Baseline and post-intervention questionnaires were administered to measure HBV knowledge, behaviors, and attitudes. Clinic vaccination records and IVR system data reports were also used to determine vaccination adherence and phone utilization, respectively. Participant satisfaction with the messages was ascertained via satisfaction surveys and telephone interviews conducted with a subsample of 12 participants.

Summary of Results: Compared to representative HBV trials (Nelson et al. 2009; Sellors et al. 1997), the overall attrition rate in our study was remarkably low for both the intervention and control groups, with >80% of participants completing the vaccination series. Results show that HBV knowledge increased 42% (p=0.015) for participants receiving the phone messages; in contrast, knowledge increased only 26% (p=0.00) for those in the control group. In terms of satisfaction, 82% of intervention participants agreed

that the appointment reminders were a good way to remember to keep vaccination appointments, and 78% agreed that they wanted to receive health education messages/appointment reminders in the future.

Conclusion: Overall, this study demonstrated that VoiceReach not only increases HBV vaccination adherence, but also improves knowledge acquisition and promotes positive attitude/behavior change among LEP patients. The System also proved to be a time- and cost-effective means to maintain provider-patient connections. VoiceReach can thus be an effective tool in improving the quality of care delivered to LEP and low FHL patients.

Product/Service being developed: VoiceReach is a multilingual, literacy-appropriate telephone care system that can help to reduce the disparity that affects individuals with LEP and/or low FHL, enabling them to access the same positive health outcomes that others have been able to attain.

49

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Canopy: Assessing the Efficacy of a Medical Spanish e-Learning System

Objective of Study: The objective of this study was to develop and evaluate the effectiveness of Canopy Medical English, our innovative web-based healthcare-focused language study program, which enables users to attain proficiency in Spanish, develop cultural awareness, and improve care for Spanish-speaking patients with limited English proficiency.

Methods: The Canopy Medical Spanish program operates on a web-based platform, removing the hassle of local installation and enabling healthcare providers to conveniently access the program. It was designed using evidence-based pedagogic methods, including audio-visual multimedia, interactive and directed instruction, frequent review and reinforcement, and flexible administration.
We conducted a four-month prospective cohort study testing 12 lessons of the Canopy Medical Spanish program. Healthcare students and professionals (N=29) with limited Spanish knowledge were recruited from two medical schools in New York City to test the software. In order to keep participants at the same pace, only 1 lesson per week was unlocked; otherwise, the program was self-paced for all users. Each lesson took approximately 60 to 90 minutes to complete.
A 73-item test was administered to participants at the study's baseline and endpoint to assess changes in cultural competency and medical Spanish proficiency. In addition, baseline and endpoint surveys (with 21 and 14 items, respectively) were administered to determine participants' self-reported Spanish proficiency and to obtain comments and suggestions for the program.

Summary of Results: The average participant test score improved by 49.5 percentage points, from 37% to 86.5%; this mean difference was statistically significant at $p<.05$ ($p=.000$). Participants had the greatest percentage gains in the areas of Vocabulary (46% gain) and Listening Comprehension (45.4% gain). The majority (82.8%) of participants reported that the program improved their cultural competency.

Overall, 93% of participants agreed that medical education programs should provide this program to students. Additionally, 75% agreed that, after using the program, their confidence in speaking, listening to, and understanding Spanish in clinical settings increased.

Main Conclusion: This interactive software promises to be an effective means for health-focused language instruction and can play a role in improving the quality of care and patient satisfaction specifically for Latino patients and patients with low English proficiency in general.

50

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Prodrugs of Neuraminidase Inhibitors for Increased Oral Bioavailability

Neuraminidase inhibitors (NI) are the most commonly prescribed class of anti-influenza drugs. However these drugs are very polar and consequently have poor oral bioavailability. Only one NI, oseltamivir (Tamiflu®), the ethyl ester prodrug of oseltamivir carboxylate (OC) has been developed as an oral drug product. Since reports of resistance to Tamiflu have increased, we sought to improve the oral bioavailability of NIs that are active against oseltamivir resistant strains of the virus. We hypothesized that the poor bioavailability could be overcome by targeting the dipeptide transporter, hpept1 or SLC15A1, which is a prevalent nutrient transporter in the intestine. Using a series of model compounds, we found that a major determinant of successful uptake was the stability of the prodrug, which, in turn, was dependent on the physical properties of the linker group of the promoiety group. We tested a series of prodrugs for intestinal drug uptake using standard in vitro uptake/transport assays (Caco-2 and hpept1 overexpressing cell lines), in situ intestinal perfusion permeability studies and standard PK preclinical studies. The final lead series of neuraminidase inhibitor prodrugs that we tested were 50 to 100 % absorbed under fed and fasted conditions. Importantly, these neuraminidase inhibitors are effective (EC_{50} of 2 to 3 nM) against neuraminidase isolated from oseltamivir resistant influenza virus. The prodrugs were shown to be effective in lethal challenge studies in mice against influenza A/NWS/33. In fact, in these studies, one NI prodrug was found to be 10 times more effective than the current marketed neuraminidase inhibitor – oseltamivir. In summary, we have developed a prodrug strategy for improving the oral absorption of two potent neuraminidase inhibitors. The prodrugs were shown to be between 50 and 100 % orally bioavailable and were as good as or better than oseltamivir in influenza challenge models. The ultimate goal of the project is to develop an oral neuraminidase inhibitor that is effective against resistant influenza strains.

51

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Ending Blindness by 2020: A Computer-based System to Screen for Eye Diseases.

Authors: P Soliz, W Bauman, C Agurto, G Zamora, and S Barriga

Objectives: To present a new system for automatic classification of digital retinal images into “normal” and “suspect” for eye disease, such as diabetic retinopathy (DR), age-related macular degeneration (AMD), and glaucoma.

Methods: Digital fundus photographs were collected from 822 patients at the Retina Institute of South Texas (RIST) and University of Texas Health Science Center in San Antonio (UTHSC-SA). The fundus photographs were: disc centered, macula centered, and superior temporal. Images were graded for the presence of three eye diseases: DR, AMD, and glaucoma. A computer algorithm based on amplitude modulation-frequency modulation (AM-FM) produced binary results on the presence or absence of pathology. A detection threshold was applied to obtain different values of sensitivity and specificity with respect to ground truth and to construct a Receiver Operating Characteristic (ROC) curve.

Results: The system achieved an area under the ROC curve (AUC) of 0.89 for detection of non-Proliferative DR and of 0.92 for detection of sight-threatening DR (STDR). With a fixed specificity of 0.50, the system's sensitivity ranged from 0.92 for all DR cases to 1.00 for clinically significant macular edema (CSME), using the presence of exudates within one disc-diameter of the fovea as a surrogate. For AMD pathologies, the system obtained a performance of AUC = 0.84 (sensitivity= 0.94, specificity= 0.50). For images that were flagged as glaucoma suspects by the human grader, the system achieved an AUC = 0.88 (sensitivity= 0.90, specificity= 0.88).

Conclusions: Of the DR pathologies studied, mild non-proliferative DR was the most challenging to detect, whereas the cases of STDR were detected with high accuracy. Although the system was not originally intended for detecting abnormalities related to AMD or glaucoma, by adding some of those cases to the algorithm's training database we were able to screen for signs of these disease with high accuracy. This work presents a viable and efficient means to characterize different retinal abnormalities and build binary classifiers for screening purposes.
The computer-aided detection algorithm based on AM-FM is being transitioned to clinical use. VisionQuest is presently collecting cases for a submission to the FDA in 2012. A network has been implemented and screening throughout New Mexico and South Texas is presently underway. In New Mexico, VisionQuest is teamed with Project Hope to conduct screening in rural areas. In South Texas VisionQuest has implemented a screening center at RIST and is screening diabetics at CommuniCare clinics in San Antonio.

Commercial Relationship: Agurto, Barriga, Zamora, VisionQuest Biomedical **E**; Baumann, Retina Institute of South Texas, **I**; Soliz, VisionQuest Biomedical, **I**.

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52

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Analyzer for Monitoring Personal Environmental Exposure to Fluids and Materials

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This project involves development and application of a low-power, compact, reliable, easy-to-operate instrument for measurement of trace elements in any liquid, gel, or solid that can be homogenized. This enables an individual environmental exposure assessment in places such as in the home, place of employment, and recreational settings. It can be used to study the personal environment of patients undergoing treatment or for individuals and for occupational health risk assessment. The goal of the project is to build a rugged, easy-to-use, field transportable analyzer and demonstrate its use for assessing exposure to multiple trace elements.

The proposed analyzer is based on a multiple monochromatic beam x-ray fluorescence (XRF) method called High Definition XRF (HDXRF). A tri-chromatic low power HDXRF system is designed and a first prototype system was built and tested. Detection limits of sub-ppm level have been achieved for Pb, As, and Hg in consumer products, liquids, and gels. A final analyzer for this project is being built with improvement to sensitivity and robustness. It will be completed in about a month and evaluated at the Wadsworth Center's Laboratories of the New York State Department of Health. A series of standards, consumer products, daily supplements, herbs and other personal related samples will be tested. Some of these data will be presented in this paper.

A significant part of HDXRF developed under this project has already contributed to publish health benefits. The U.S. Consumer Product Safety Commission just recently approved the use of ASTM 2853, an HDXRF method for testing Pb in paint in children's and consumer products.

53

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A High-Performance Breast CT System for Dedicated Breast Imaging

Objective: The objective of the SBIR project is to develop and perform an initial patient pilot study of a next generation dedicated breast CT scanner which provides improved lesion detection as well as improved patient experience through the elimination of compression.

Methods: A novel next generation system was developed and fabricated which provides unique 3D complex trajectories for improved sampling and improved access to the full breast and chest wall. A custom designed and specially contoured patient bed was also developed for facilitating improved access to the chest wall while maintaining patient comfort, thereby minimizing patient motion. Alongside the hardware development, a new user interface has been developed as well as new multi-threaded acquisition software for improved performance and synchronization. A system overview of components and performance is provided. The CT system includes a CsI digital flat-panel detector used with both full resolution (197 micron pixels) and 2x2 binned mode (394 micron pixels) resolution, as well as a single pole x-ray tube. The X-ray tube is matched to a generator that is capable of providing fast repeated exposures up to 30 frames per second with less than 4 ms rise times. The system has a fixed SID, and precisely controlled polar, azimuthal, and vertical motions allowing an unlimited number of arbitrary complex trajectories. Volumetric sampling elimination of cone beam distortion artifacts is evaluated with Defrise disk phantoms. The 3D MTFs are evaluated. CT quantification with software based scatter and motion correction is performed. A novel CPU-based statistical reconstruction is employed.

Results: The system has been shown to perform accurate 3D step and shoot scans at under 60 seconds per breast. The objects are reconstructed without distortion using iterative reconstruction. Motion artifacts are eliminated. Reconstructions perform well at 3.5 minutes per reconstruction for 2x2 binned data.

Conclusion: This high performance, dedicated breast CT system meets performance expectations and is ready for patient acquisitions.